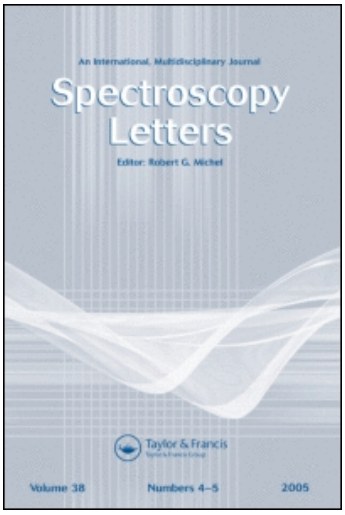

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FAB MASS SPECTRA OF PEPTIDES, PART IX. FORMATION OF n H_3PO_4 AND n H_3PO_4 + 62 ADDUCTS ON H_3PO_4-SPIKED GLYCEROL MATRICES

Key-words: FAB-MS, Peptides.

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ABSTRACT

We have studied the behaviour of the brain pentapeptide leucine-enkephalin on phosphoric acid spiked glycerol matrices under FAB-PI conditions. Ion series of the general formula $[mM+nH_3PO_4+H]^+$, $[mM+H+62]^+$ *and* $[mM+H_3PO_4+H+62]^+$ *were observed. Experiments using labelled glycerol, methionine-enkephalin and various spiking agents were performed along with B/E linked scans in order to investigate the nature of the latter two series of ions; they led to the conclusion that the +62 ions originate from the slow decomposition of* H_3PO_4*-peptide aggregates.*

INTRODUCTION

As part of our general work on FAB mass spectra of peptides, we reported earlier on the formation and structure of protonated dimer adducts, $[2M+H]^+$, for the brain pentapeptide, leucine-enkephalin[1-3], **1**.

H-Tyr-Gly-Gly-Phe-Leu-OH

1

Several conclusions were drawn from these results, one of which was related to the opportunity for **1** to form helicoidal-like or *beta*-pleated (*beta*-sheet) adducts in order to account for the observed decomposition of the dimer adducts[2,3]. The protonated dimer $[2M+H]^+$ or trimer $[3M+H]^+$ ions could result from the fragmentations of these polymeric aggregates as well as from the spontaneous di- or trimerisation onto the glycerol matrix of the monomeric peptide **1**. Tetra- or pentameric ions are not detected in the mass spectrum of this compound because of their low intensity at such a high mass range. However, data obtained by varying the concentration of the peptide in glycerol, and the basic characteristics of the solvent (*e.g.* dielectric constant) along with our previous dipeptide results (especially on the formation of mixed dimers under FAB, CI or DCI conditions[4]) strongly support the hypothesis of the formation of higher aggregates from the monomeric **1** onto glycol, glycerol or thioglycerol matrices.

To confirm this hypothesis we have designed a series of experiments aimed at evaluating the affinity displayed by the peptides toward phosphoric acid and toward selected polyamines. The latter was presented in another

TABLE 1

Characteristic Ions and Ion-Types Observed in the FAB Mass Spectra of Leucine-Enkephalin Recorded on a Phosphoric Acid-Spiked Glycerol Matrix

m/z	Ion Type
556, 654, 752, 850, 948, 1046, 1144	$[M+H+nH_3PO_4]^+$; n=0-6
618, 716, 814	$[M+H+n\ H_3PO_4\text{-}2H_2O]^+$; n=1-3
1112, 1210, 1308	$[2M+H+nH_3PO_4]^+$; n=0-2
1174, 1272	$[2M+H+nH_3PO_4\text{-}2H_2O]^+$; n=1,2

report[5]. These experiments are part of our ongoing investigations aimed at the design of biointeractive matrices for structural work in FAB-MS.

RESULTS AND DISCUSSION

The $[M+H+glycerol]^+$ as well as the $[2M+H+glycerol]^+$ adducts have been observed in the spectra of a whole variety of peptides. In the case of **1**, $[M+H]^+$, $[2M+H]^+$ as well as $[3M+H]^+$ ions were also detected. When phosphoric acid (H_3PO_4), **P**, is used to spike a glycerol matrix containing **1**, four series of new adduct ions can be observed. They are listed in Table 1.

The amide (peptide) bond-phosphoric acid affinity should justify the formation of [polyphosphate-peptide] or [n(phosphoric acid)-peptide] adduct ions in the first series of ions at m/z 556, 654, ..., 1144. Each amide bond of **1** reacts with **P** accounting for the addition of the first four phosphoric acid residues (nominal value of 98 each) with a fifth phosphoric acid being fixed

onto the terminal amine. For this particular substance, a sixth phosphoric acid unit is fixed on the phenolic OH group of the tyrosine residue. The addition of n x 98 daltons to the protonated peptide ions is a clear indication of a H-bond between **P** and the peptide rather than a polyphosphate-peptide association.

Another closely related series of ions involving the $[2M+H]^+$ species are also observed at m/z 1112, 1210 and 1308. Furthermore, both original ions, $[M+H]^+$ and $[2M+H]^+$ are followed by significant adduct ions[7] at $[M+H+62]^+$ and $[2M+H+62]^+$ and at $[M+H+62+P]^+$ and $[2M+H+62+P]^+$. FAB positive ion spectra recorded in glycerol that was spiked with other mineral acids or source of protons than **P** (*e.g.* HCl, H_2SO_4, *m*-nitrobenzyl alcohol, phenol) did not contain these artefacts. A possible explanation for the origin of the +62 daltons aggregates is the decomposition of glycerol, *via* hydrogen shift, producing an ethylene glycol adduct. Phosphoric acid appears to play an important role in generating this adduct and it also adds on to the "+62 adduct" ions mentioned above to yield the "+62+P" aggregates.

In order to test this hypothesis, spectra of **1** were recorded on ethylene glycol and 1-thioglycerol matrices in the presence of phosphoric acid. If the assumption is correct, methanol and either ethylene glycol, 2-mercaptoethanol or a combination of both adducts should be observed, respectively. Ethylene-glycol spectra do not show any methanol adducts ($[M+H+32]^+$). However, the high volatility of the methanol so-produced could account for its non-detection. The 1-thioglycerol-phosphoric acid spectra of **1** display intense adduct ions at $[M+H+62]^+$ and $[2M+H+62]^+$

as well as some **P** adduct to both ions but no $[M+H+78]^+$ and no $[2M+H+78]^+$ ions. Furthermore, the phosphoric acid spiked matrices of polyethylene glycol-300 and 1,3-propanediol do not show any important adducts. If they are matrix-related, it would appear then that the presence of a *vic*-diol system is a prerequisite for the formation of these uncommon adducts.

The use of perdeuterated glycerol (glycerol-d_8), instead of non labelled glycerol leads to the deuteration of peptide (both pseudomolecular ion cluster and +62 dalton adduct ions shifted by +4 daltons at 560 and 622 daltons). Experiments with C-deuterated glycerol (glycerol-d_5) does not confirm these results even after four consecutive reprotonations ofthe solvent.

Substituting methionine-enkephalin for leucine-enkephalin gives rise to analogous results ($[M+H+62]^+$ at m/z 636). We have tested for other potential +62 dalton unit sources such as copper ions ($CuCl_2$, $CuSO_4$, metallic copper wire), as well as the mixture of sodium and potassium chlorides, but to no avail. In the last case, however, a new series of adduct ions are formed as the result of direct cationization of the peptide (*e.g.* $[M+alkali]^+$, *etc.*). Finally, collision experiments on the ion at m/z 618 lead, as expected, to the intense pseudomolecular ion.

The use of concentrated H_3PO_4 alone to spike the glycerol matrix does not allow the observation of the glycerol adduct. The analysis of mixture on a cold tip (liquid nitrogen) revealed only slight amount of the glycol adduct without HCl spiking. The spiked matrix (glycerol+HCl+P) background

spectrum does not show any 618 or 1174 ions in absence of peptide. Furthermore, in all experiments with phosphoric acid spiked matrices, the presence of a doubly charged ion at m/z 609 was observed (see acknowledgments). Thus, it would appear that the +62 aggregates originate from the slow decomposition of H_3PO_4-peptide adduct ions themselves.

In order to confirm this last hypothesis, we performed a series of B/E linked-scanning experiments on the m/z 654. The data obtained from these B/E spectra showed that this particular ion, namely $[M+H_3PO_4+H]^+$, decomposes to $[M+H_3PO_4+H\text{-}H_2O]^+$ and to $[M+H]^+$ at m/z 636 and 556, respectively. Similar experiments performed on the ion at m/z 636 yielded a daughter ion at m/z 618 ($[M+H_3PO_4+H\text{-}2H_2O]^+$). Finally, B/E spectra recorded from the ion at m/z 618 showed that the latter had daughter ions at m/z 600 ($[M+H_3PO_4+H\text{-}3H_2O]^+$) and again at m/z 556. These data provide us with the origin of the +62 ions that complicated greatly the original interpretation of the spectra. This behaviour is rather peculiar although it complements the data we reported earlier on polyamines binding[5].

EXPERIMENTAL

Spectra were obtained on VG-70EQ, Nermag-3010 and Finnigan MAT-312 mass spectrometers using their respective FAB gun and stainless steel and copper tips. Operating conditions included glycerol, thioglycerol or ethylene glycol as support matrix spiked with 2-10% of H_3PO_4 (**P**) (analytical grade) and research grade xenon gas as atom source. The peptide was suspended in the matrix and subsequently treated with a small amount of diluted H_3PO_4 (*ca.* 2%) and spiked with HCl (3M). The glycerol-d_8 (DMM 123, C. E. A., Saclay, France) was analysed as 98% pure[3,6]. The peptides

were obtained commercially from Sigma Chemicals. Copper wire, $CuCl_2$, $CuSO_4$, KCl and NaCl were of reagent grade and used as obtained (Fisher).

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