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ON THE INFLUENCE OF HYDROPHOBICITY IN THE SIMS SPECTRA OF AMINO ACIDS IN GLYCEROL MATRIX

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Mass spectrometry is becoming an important method in structure determination of biopolymers. Determination of the nature and the number of the various monomers is an important information for the elucidation of fragmentation patterns. We present here a matrix designed to allow both identification and semi-quantitative measurement of amino acids residues in peptides.

Mass spectrometry of low volatility and labile compounds receives an increased attention since the introduction of liquid matrices for SIMS and FABMS (1). One of the most growing field of application is the sequencing of biological polymers of which peptides are an important group (2). Besides the sequence information which, although often incomplete, is contained in the SIMS-FAB spectra of underivatized peptides, a rapid semi-quantitative survey of the amino acid residues after hydrolysis of the parent molecule is of valuable interest. For this purpose, direct semi-quantitative comparison of amino acids $[M+H]^+$ signals in a single spectrum has to be possible if the slow chromatographic step has to be avoided.

We present here a suitable matrix composition and operation conditions for that purpose.

EXPERIMENTAL SECTION

SIMS spectra were taken with a previously described quadrupole system (extranuclear 7-162-8) and a Cs^+ ion gun (3).

The amino acid solutions were prepared by dilution of a weighted stock solution. The solutions were acidified to pH = 1 by HCl addition. Twenty microliters of solution were mixed on

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the copper probe with twenty microliters of glycerol containing the various sulfonic acids. The samples were pre-evacuated (elimination of water and HCl) and then introduced in the ionization chamber. The spectra were obtained with 5 KeV Cs^+ ions. Two hundreds scans of 0.4 sec each (0-600 amu) were accumulated in a multichannel analyser and the complete procedure was repeated three times to check the reproducibility of results. The measured current on the target was kept at 0.5 μA and all pumping times were kept constant to avoid differences in glycerol evaporation. The samples were examined after bombardment to check the absence of precipitation or visible damages.

RESULTS AND DISCUSSION

After an extensive study of the behaviour of single amino-acids in glycerol solutions, we concluded (4) that their hydrophobicity is an important parameter for the analysis of SIMS-FAB sensitivity provided they are all in the protonated form (acidic solutions).

As an example, we show in fig. 1, the relation between the $(M+H)^+$ signal normalized against the $(G+H)^+$ one for different concentrations of the amino acid (M).

Taking into account the importance of the hydrophobicity, we can look for an appropriate promoting acid which, instead of being only a proton donor could also suppress the matrix containing peaks and would thus offer a large spectral window without any interference.

Sulfonic acids were chosen for their low vapor pressure, small pK_a values (below 1) and good solubility in glycerol (5). Their surfactant properties increase when going from methanesulfonic to paratoluenesulfonic and camphorsulfonic acids.

We show, in fig. 2, the positive SIMS spectra of the same Leucin solution without a promoting acid (a) and with the different sulfonic acids (b,c,d).

The absolute intensity of the pseudomolecular ion peak increases when increasing the hydrophobicity of the sulfonic acid. At the same time glycerol containing peaks and amino acid dimer

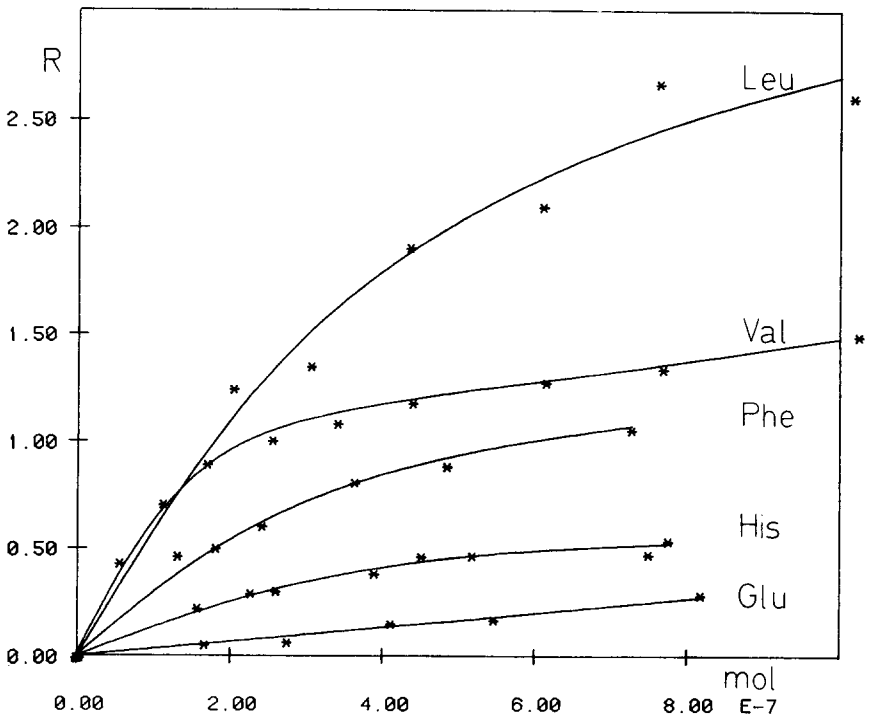

Fig. 1. Relation between the ratio $R = I_{[M+H]^+}/I_{[G+H]^+}$ and the amino acid (M) quantity for various amino acids.

peak vanish whereas $[M+A+H]^+$ and $[2M+A+H]^+$ peaks increase significantly. According to the molecular mass of camphorsulfonic acid, a spectral window of 232 mass units is available for the detection of all the amino acids without interferences.

As regards the mechanism of promotion of the molecular ion, we assume that the hydrophobic sulfonic acid migrates towards the surface of the glycerol droplet as a neutral molecule or as an anion. It can there either protonate a hydrophobic amino acid owing to its strong acidity or "pump" the positively charged hydrophilic amino acid when the acido-basic reaction proceeded in the bulk of the solution. The increase of $[M+A+H]^+$ ion signal parallel to the hydrophobicity of the promoting acid supports the close vicinity of both molecules during the ion formation process.

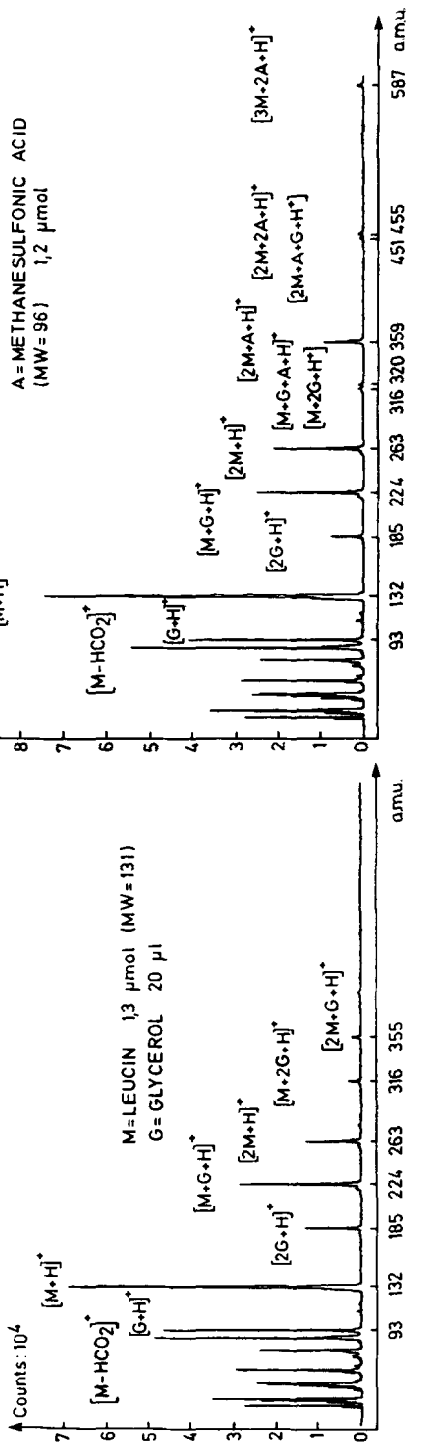

Fig. 2. Evolution of the SIMS spectrum of a Leucin solution (1,3 µmol in 20 µl of glycerol) when changing the hydrophobicity of the promoting agent.
a) no acid b) methanesulfonic acid (1,2 µmol) c) paratoluenesulfonic acid (1,4 µmol)
d) camphorsulfonic acid (1 µmol)

TABLE 1

AMINO ACID	A MEASURED QUANTITY ($\times 10^{-9}$ mol/20µl)	B WEIGHTED QUANTITY ($\times 10^{-9}$ mol/20µl)	ERROR $\frac{A-B}{B} \times 100$
Leu	248	252	-1,6
Val	197	282	-30
Phe	164	188	-13
His	465	239	+95
Tyr	116	221	-48
Gln	281	342	-18

In order to test the possibility of application of this promoting agent to amino acids mixtures, we made a single spectrum of a sample composed of six amino acids in the sub-micromol range. The results derived from the spectrum are compared in Table 1 with the known amounts of amino acids.

As can be seen from data presented, the agreement between the real and measured values, even imperfect, should be sufficient to establish the peptide composition when coupled with the molecular weight knowledge. This, in turn, is very useful to interprete the fragmentation pattern of SIMS and FAB spectra. The calibration curves obtained with our instrument are valid down to 5 nanomoles of amino acid on the sample support. Changing for the smaller probes available with commercial instruments (1 mm^2) would allow a 10 to 50 times decrease in sample consumption.

Discrepancies between measured and actual amino acids quantities, due to enhancement or suppression of some components of the mixture are smaller than those obtained with pure glycerol. Then some peaks can even be completely suppressed. A full description of the phenomenon is under study.

In conclusion, we show here that a glycerol solution of camphorsulfonic acid is a good matrix for peptide SIMS/FAB work. In fact it has not only a good spectral window but also it decreases the mutual influences of the sample's components allowing semi-quantitative analysis.

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