ANALYSIS OF MIXTURES OF SMALL PEPTIDES

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SUMMARY The identification of dipeptides in mixtures was aided by their conversion into 7-isopropyl-1-methylazulen-4-ylacetyl or 1-naphthylacetyl derivatives followed by mass spectrometry of the mixtures. Dipeptides in urine were identified more completely than was possible by gas chromatography - mass spectrometry alone. Similarly, analysis of dipeptides resulting from enzymolysis of an oligopeptide was achieved readily at high sensitivity.

INTRODUCTION In earlier work (1), the urine of a patient suffering from dermatological purpura was examined for dipeptides by gas chromatography - mass spectrometry after conversion of the dipeptides into their N-acetyl, carbomethoxyl derivatives (1, 2). All the identified dipeptides contained proline as the second amino acid residue and the distribution of the constituent amino acids was consistent with breakdown of collagen. The gas chromatograms were complex with many minor, often overlapping

peaks and, although the major dipeptides were identified, dipeptides present in lower abundances gave mass spectra which were ambiguous. The reasons for this were the presence of ions other than sequence ions from the peptides themselves, the generally low abundances of molecular ions, the presence of amino acids as well as dipeptides, and ions from 'background' substances. These problems are common in investigations of peptides from natural origins.

The interpretation of the mass spectra of peptides (especially in mixtures) is troublesome when molecular ions cannot be identified. Molecular ions of peptide derivatives are often not pronounced after electron-impact ionization and, in such situations, chemical or field ionization are useful for the analysis of mixtures (3). We have found that with two similar types of N-protecting group, carbomethoxylated peptides gave abundant molecular ions and mixtures of peptides were readily analysed; the two groups are 7-isopropyl-1-methylazulen-4-ylacetyl (IMAA) which has the added advantage of making peptides blue and readily visible on chromatography (4), and the more stable 1-naphthylacetyl (NA).

MATERIALS AND METHODS Peptides were carbomethoxylated by reacting them with 0.3M HCl in methanol at 20° for 24 hr. After addition of a slight excess of triethylamine, the carbomethoxylated peptides were reacted with the N-hydroxysuccinimido ester of 7-isopropyl-1-methylazulen-4-ylacetic (4) or 1-naphthylacetic acid in dioxan-water (7 : 1) at 20° for 14 hr. The solvent was evaporated and the sample placed on the direct inlet probe of the mass spectrometer. Any small excess of active ester, being volatile, could be pumped off before the spectra of the

N-acylated, carbomethoxylated peptides appeared. Spectra of the latter were obtained by electron impact at source temperatures of 180 - 230°. Mass spectra were recorded at intervals as the temperature increased; at the lower temperatures, mass spectra of the protected amino acids and lower molecular weight dipeptides were obtained whilst, at higher temperatures, mass spectra of the less volatile peptides were obtained.

Active esters were prepared from N-hydroxysuccinimide and the appropriate acid in the presence of dicyclohexylcarbodiimide in the usual way (5).

The dodecapeptide, H.glu.ala.glu.glu.glu.ala.tyr.gly.trp.met.asp.phe.OH (3 mole), was treated with dipeptidylaminopeptidase I (6) to give a mixture of dipeptides. The latter were converted into their IMAA or NA carbomethoxylated derivatives and mass spectra run on the mixture. All the expected dipeptides gave abundant molecular ions and, for the NA derivatives, sequence ions were observed except for trp.met. Edman degradation (7) on another sample of the dodecapeptide followed by digestion with dipeptidylaminopeptidase I gave a second lot of overlapping dipeptides, identified as their NA derivatives; the NA derivative of the C-terminal phenylalanine residue also gave an abundant molecular ion.

The dipeptides from the crude mixture from urine (1) were converted into IMAA, carbomethoxylated derivatives and analysed by mass spectrometry.

Mass spectra were obtained at 70eV on an AEI/MS 12 mass spectrometer with a direct insertion probe.

RESULTS AND DISCUSSION The IMAA, carbomethoxylated peptides from urine (1) gave abundant molecular ions corresponding to the dipeptides, R.Pro (R = gly, ala, val, leu, ile, phe, asp, asn,

glu, tyr, ser, and thr). With this information, the gas chromatographic - mass spectrometric traces from the earlier investigation (1) were re-examined and many of the minor components identified. No dipeptides were observed which did not contain proline.

The high sensitivity of the method led us to investigate its use further by examining the mixture of peptides obtained by the action of dipeptidylaminopeptidase on a dodecapeptide, H.glu.ala.glu.glu.glu.ala.tyr.gly.trp.met.asp.phe.OH; the dipeptides obtained were converted into their N-IMAA or N-NA, carbomethoxylated derivatives and examined by mass spectrometry. Molecular ions were readily detected for all the expected dipeptides (Table). It is possible to determine the sequence of amino acids in a peptide without knowing the actual order of amino acids in the individual dipeptides (8). To do this, it is necessary to remove a terminal amino acid from the original oligopeptide and repeat the enzymolysis and mass spectrometry on the resulting

TABLE. Dipeptides from enzymolysis of a dodecapeptide and an undecapeptide.[a]

Dodecapeptide		Undecapeptide	
Dipeptide found	Molecular ion[b]; m/e	Dipeptide found	Molecular ion[b]; m/e
glu.glu	486	ala.glu	414
ala.glu	414	glu.glu	486
tyr.gly	420	ala.tyr	434
trp.met	517	gly.trp	443
asp.phe	476	met.asp	460
		phe[c]	347

[a] The dodecapeptide was examined knowing only the amino acid composition, ala_2, glu_4, gly_1, tyr_1, trp_1, met_1, asp_1, phe_1; of the 30 dipeptides possible from this composition, only five were found; the undecapeptide was obtained from N-terminal degradation of the dodecapeptide.

[b] NA, carbomethoxylated derivatives.

[c] C-terminal amino acid from enzymic digest.

des-oligopeptide. This was done with the dodecapeptide giving the second series of dipeptides shown in the Table. From the two sets of results in the Table it is possible to reconstruct the original sequence of amino acids in the dodecapeptide (8).

The simplicity and high sensitivity of this method would also make it useful for the 'time-elapsed' method of peptide sequencing in which the ordering of amino acids in a chain is determined by the order of appearance of dipeptides as enzymolysis proceeds (9). The NA derivatives would be particularly useful in such a method because they give sequence information from fragment ions as well as abundant molecular ions.

CONCLUSION Analysis of mixtures of simple peptides can be carried out rapidly and at high sensitivity by mass spectrometry of IMAA or NA derivatives which give abundant and clearly recognisable molecular ions. Generally, the IMAA derivatives are volatile and show very little fragmentation of the molecular ions, making examination of mixtures particularly easy. The NA derivatives are also volatile but also give sequence information in a relatively few fragment ions. The method has been demonstrated by a more complete analysis of amino acids and dipeptides in urine than was possible by gc-ms alone and by its applicability to amino acid sequencing in an oligopeptide.

References

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