PEPTIDE SEQUENCING BY LOW RESOLUTION MASS SPECTROMETRY

I. THE USE OF ACETYLACETONYL DERIVATIVES TO IDENTIFY N-TERMINAL RESIDUES

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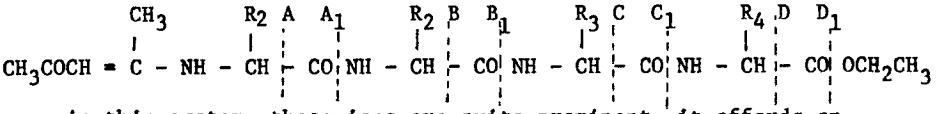
The mass spectrometric sequence determination of oligopeptides via their acetylacetonyl derivatives gives reliable results with small peptides (2-10 residues) regardless of the amino acids present. The main advantages are the ease of derivative formation and the mass spectral fragmentation patterns which are optimal for N-terminal amino acid identification.

Many published methods for sequence determination of peptides which use,manually interpreted, low resolution mass spectra, depend on a recognition of the molecular ion and a search for ionized amide bond cleavages (1-5). Since an unambiguous identification of a small molecular ion peak in a mass spectrum is often difficult, sequencing procedures based on a recognition of the more prominent N-terminal amino acyl ion are to be preferred. Whilst mixtures of long chain fatty acids or CH_3CO and CD_3CO (6) have been used for distinctive N-terminal tags for low resolution mass spectrometry of peptides, all automated procedures have relied on the recognition of elemental composition of the N-acyl fragment by high resolution mass spectrometry. (7,8) In view of the high cost of this instrumentation, we have re-examined the sequencing of peptides by low resolution mass spectrometry with derivatives which are optimal for N-terminal amino acid identification. We now report on the sequencing of acetylacetonyl peptides (ACA-peptides), which gives reliable results with small peptides (2-10 amino acid residues), regardless of the amino acids present. The method depends on the identification of the N-terminal amino acid residue 'A' in the spectra.



Since, in this system, these ions are quite prominent, it affords an unambiguous starting point for the search for the sequence ions (B, C, D and B_1, C_1, D_1). Preparation of the volatile derivatives involves esterification of the peptide followed by treatment with acetylacetone. Under the experimental conditions used, arginine peptides are converted to δ-N-(2-pyrimidinyl) ornithine peptides (9) and the ε amino group of lysine is also derivatised. All other functional groups present in the protein amino acids remain intact and are left unprotected. A typical sample preparation involves refluxing the peptide (0.5-1.0 mg) with dry ethanolic hydrogen chloride (1 ml, 25-30% w/w) for 10 min and removing the solvent in vacuo. After redissolving in dry ethanol (100 µl), acetylacetone (30 µl) and dry ion exchange beads (AG1-X8, Bio-Rad Cal., in the bicarbonate form) are added till the solution is at pH 7-8. Molecular sieve (3A, Matheson, N.J.) is added to remove traces of water and the reaction mixture is left overnight. The supernatant liquid is then withdrawn with a syringe and the solvent evaporated in vacuo. The residue is then inserted directly into the ion source of a low resolution mass spectrometer. On the basis of the mass spectra of almost 80 model peptides, we find that the "A" fragment is the most prominent peak in the spectra of ACA-peptide esters, which have an N-terminal aliphatic- or acidic-amino acid. (Table 1) In some cases partial loss of the side chain is observed with N-terminal methionine, serine, threonine, aspartic and glutamic acid. Some larger seryl- and threonyl-peptides tend to dehydrate at higher probe temperatures and this makes recognition of these residues in the N-terminal position difficult. Histidyl-, phenylalanyl-, tyrosyl- and tryptophyl-peptides show some elimination of the side chain as $ArCH_2^+$ or $ArCH_2^\bullet$, but these ions help to confirm the presence of these residues. Lysyl and arginyl peptides yield the very characteristic [A-99] fragments, and show only very small "A" fragments in the mass spectra (Figure 2), whilst cystine

Table 1

Characteristic Fragments Used for Identification of N-Terminal Amino Acids

N-Terminal Amino Acid	"A" Fragment (m/e) $[CH_3-CO-CH=C(CH_3)-NH-CHR]^+$	Other Ions (m/e) Used for Identification
GLY	112	
ALA	126	
SER	142	112 $(A-CH_2O)^+$
PRO	152	
VAL	154	
THR	156	138 $(A-H_2O)^+$, 112 $(A-CH_3CHO)^+$
LEU	168	
ILE	168	
HYP	168	150 $(A-H_2O)^+$
ASN	169	
MET	186	138 $(A-CH_3SH)^+$
HIS	192	
ASP	198	
PHE	202	
GLU	212	
TYR	218	
TRY	241*	130 $(Ar-CH_2)^+$
LYS	265*	166 $(A-NH_2-C(CH_3)=CH-COCH_3)^+$
ARG	275*	176 $(A-NH_2-C(CH_3)=CH-COCH_3)^+$
CYS	-	156 $(A/_2-H)^+$, 158 $(A/_2+H)^+$

*Minor fragment.

derivatives undergo S-S bond rupture accompanied by hydrogen transfer (10). Although peptides containing unmodified asparagine have been sequenced, diazomethane has to be used instead of alcoholic hydrogen chloride for esterification (11) to prevent hydrolysis to the corresponding aspartyl peptide. The ACA-peptide esters have a relatively high vapor pressure and yield readily interpretable mass spectra from hepta- and octa-peptides containing only the neutral amino acids (Figure 1). The presence of basic polyfunctional amino acids decreases the volatility and limits the sequencing procedure to tetra- and penta-peptides. Experiments to increase the volatility of the ACA-peptide esters by permethylation (12-15) invariably yielded a complex mixture of products. The potential difficulty is the enamino ketone function which reacts with methyl iodide to give O and C alkylation (16) and a non volatile quaternary salt.

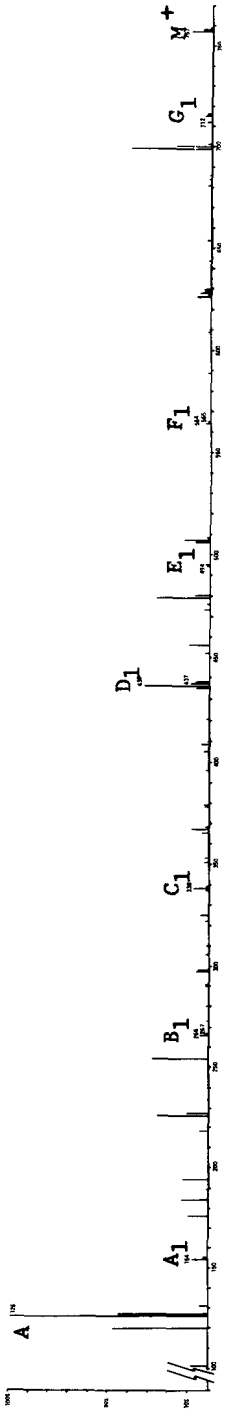


Figure 1. Mass Spectrum of ACA-Ala-Leu-Ala-Val-Gly-Ala-Phe Ethyl Ester
(Atlas CH 4 Mass Spectrometer, Temperature 260° C)

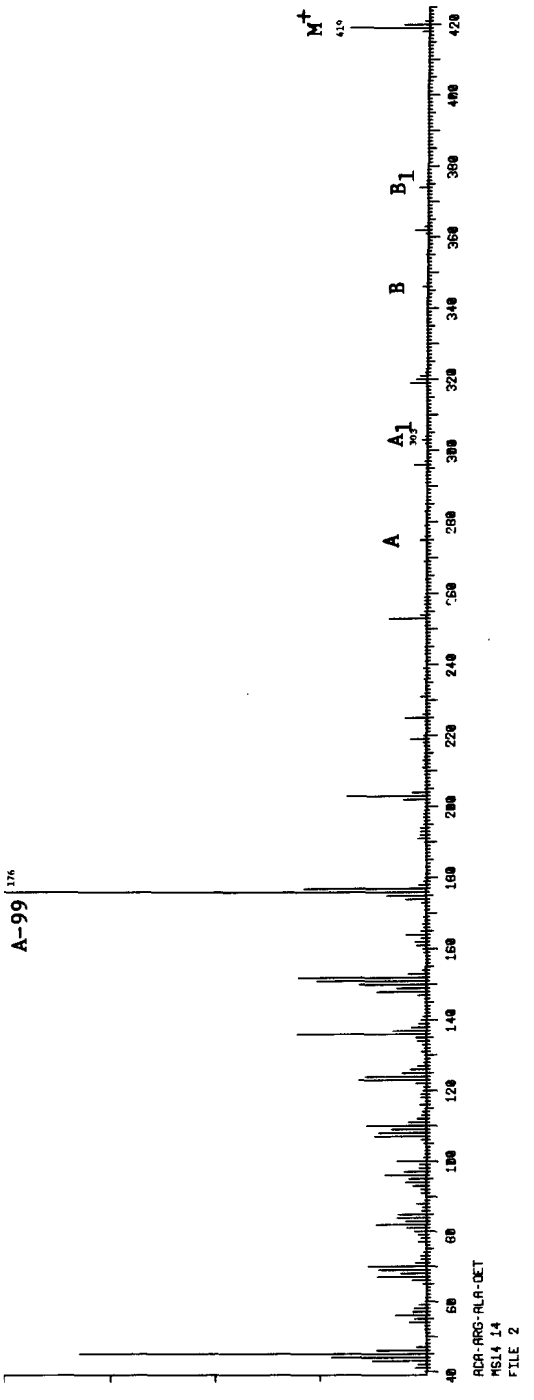


Figure 2. Mass Spectrum of ACA-Arg-Ala Ethyl Ester
(Finnigan 1015 Mass Spectrometer)

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