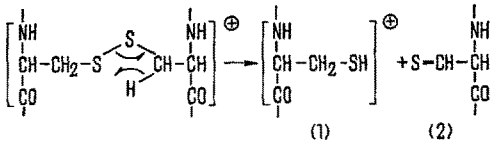
Mass Spectrometric Determination of the Amino Acid Sequence in Cystine- and Cysteine-Containing Peptides

We have previously shown that the amino acid sequence in peptides can be determined by analysis of the mass spectra of their *N*-acyl derivatives (see [1,2] and references therein). Such analysis was based on the amino acid type of fragmentation and it was pointed out that rapid, unequivocal deciphering of the spectra required preliminary knowledge of the amino acid composition of the peptides and of the fragmentation behaviour of all the constituent amino acid residues. In the light of this we undertook a study of the mass spectrometric behaviour of methyl esters of cystine- and cysteine-containing *N*-acyl peptides.

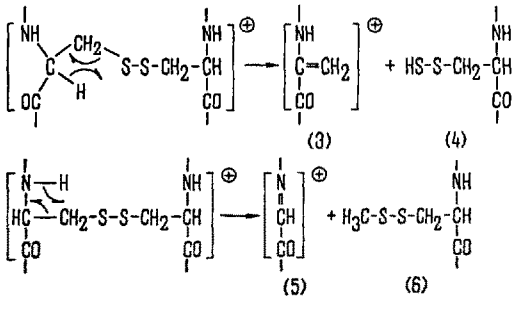
The mass spectrum of only 1 cystine peptide has been described in the literature: LEDERER and co-workers[3] observed in the mass spectrum of dicarbobenzoxyglycylcystine methyl ester, a molecular peak (m/e 650) and also peaks with m/e 324 and 326. According to our data, molecular peaks are present only in the case of dipeptides. Under the mass spectrometric conditions, higher molecular weight compounds of this series very easily undergo S-S bond rupture accompanied by transfer of a hydrogen atom from the neutral half to the charged half of the split molecule:



(1) (2)

Hence the mass spectra of esters of cystine-containing *N*-acylpeptides are actually those of the corresponding cysteine derivatives with unprotected SH groups (see Figure 1).

Under the mass spectrometric conditions, the cystine residue in the peptides undergoes fission not only at the S-S bond, but also at the C_β-S and C_α-C_β bonds, both processes being accompanied by transfer of a hydrogen atom:

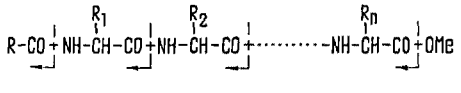


(3) (4)

(5) (6)

It should be stressed that all 3 processes largely proceed with the positive charge localized on fragments of type (1), (3) and (5); concurrently, ions (3) and (5) can form from ion (1) by elimination of H_2S or CH_3SH_1 respectively.

All 3 ion types undergo further fragmentation of the amino acid type[1,2] involving consecutive rupture of the amide bonds so that the positive charge is localized on the *N*-protected fragment:



Because of this in the mass spectra of cystine-containing peptides peaks (Figure 1, m/e 606, 575, 518, 490, 405, 258 and 230) due to amino acid fragmentation of type (1) ions are usually accompanied by peaks lower by 34 and 48 m.u., corresponding to fragments of type (3) and (5) (Figure 1, m/e 572, 484, 470, 456, 371, 357, 343 and 224). Obviously, identification in the mass spectra of cystine-containing peptides of peaks corresponding to the amino acid fragmentation of ions (1), (3) and (5) is a sufficient condition for determining the amino acid sequence of such peptides.

In the present study we confined ourselves to only symmetrical cystine peptides. The mass spectra of unsymmetrical cystine peptides will evidently be the sum of the mass spectra of 2 different peptides. Their analysis in terms of the amino acid type of fragmentation, while difficult, is quite possible (cf. BIEMANN et al.[4] on the analysis of the mass spectra of peptide mixtures).

Regarding the mass spectrometric behaviour of cysteine derivatives, the literature contains information only on that of S-benzylcysteine peptides. MCLAFFERTY[5] has observed the presence of Z-NH-$CH(C_4H_9)$-$C{\equiv}O^{\oplus}$ and Z-$\overset{\oplus}{N}$H=$CH(C_4H_9)$ ions in the mass spectrum of Z-Ile-Cys(Bzl)-Ser-OMe, but other characteristic peaks could not be found in the mass spectrum of this compound. BAYER et al.[6] studied the mass spectra of the esters of *N*-trifluoroacetyl derivatives of a number of S-benzylcysteine peptides. These authors found the characteristic peaks to differ depending upon the position of the S-benzylcysteine residue in the peptide chain, but did not evolve a general method for determining the complete amino acid sequence in such peptides.

In the present investigation our main purpose was to elucidate the mass spectrometric behaviour of peptides such as could be expected to form in partial protein hydrolysis and to develop a mass spectrometric method for determining their amino acid sequence. From this point of view it would have been quite pointless to work with S-benzylcysteine derivatives, these being only intermediates in the synthesis of cystine- and cysteine-containing peptides. Because of the tendency of cysteine-containing peptides to undergo oxidative condensation, they are practically not employed as such without protection of the SH groups in the structural chemistry of proteins. Usually the thiol groups of cysteine residues in proteins are blocked by alkylation or oxidation. We therefore selected for study primarily cysteic acid, S-β-aminoethylcysteine and S-carboxymethylcysteine peptides, the most widely used and most stable of the cysteine derivatives.

N-Acylpeptide esters with cysteic acid residues are highly volatile. Despite this it is difficult from their mass spectra to obtain information on their amino acid sequence. Apparently due to the thermal instability and

[1] M. M. SHEMYAKIN, YU. A. OVCHINNIKOV, A. A. KIRYUSHKIN, E. I. VINOGRADOVA, A. I. MIROSHNIKOV, YU. B. ALAKHOV, V. M. LIPKIN, YU. B. SHVETSOV, N. S. WULFSON, B. V. ROSINOV, V. N. BOCHKAREV and V. M. BURIKOV, Nature *211*, 361 (1966).

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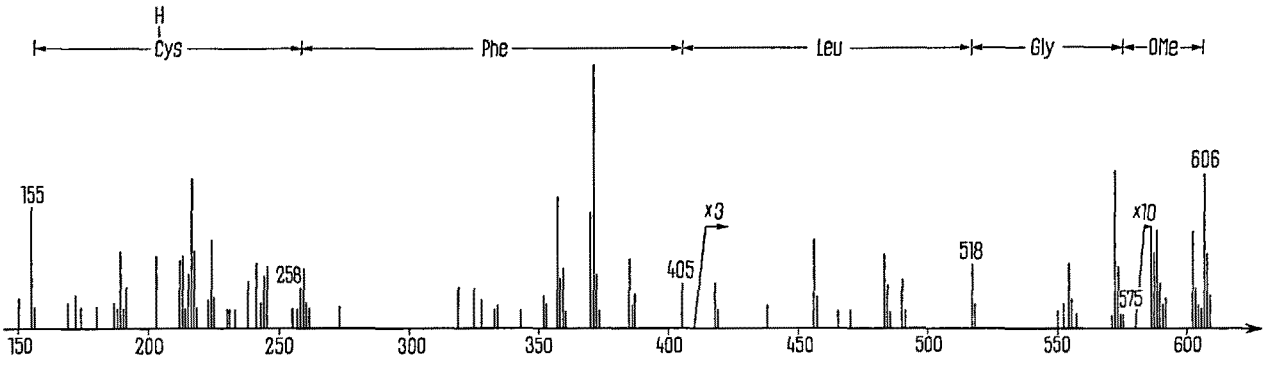


Fig. 1. Mass spectrum of (Dec-Cys-Phe-Leu-Gly-OMe)$_2$.

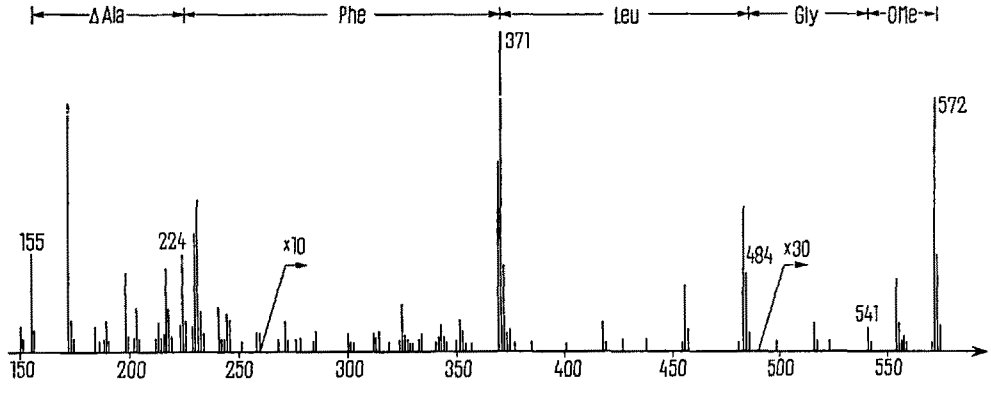


Fig. 2. Mass spectrum of Dec-Cys(CH_2CH_2NHDec)-Phe-Leu-Gly-OMe.

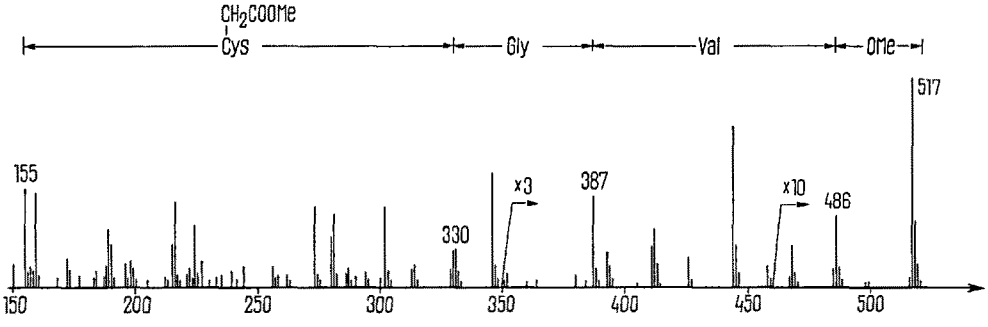
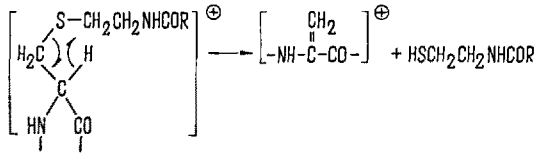


Fig. 3. Mass spectrum of Dec-Cys(CH_2COOMe)-Gly-Val-OMe.

to localization of the positive charge on the SO_3CH_3 group such peptides suffer random fission of the peptide chain. In this respect they are similar to the esters of $N^{\alpha}, N,^{G} N^{G}$-triacyl derivatives of arginine peptides[2].

Methyl esters of S-β-aminoethylcysteine-containing $N^{\alpha}, N^{\varepsilon}$-diacylpeptides under mass spectrometric conditions very readily eliminate the acylaminoethyl mercaptane, transforming into derivatives of dehydroalanine:

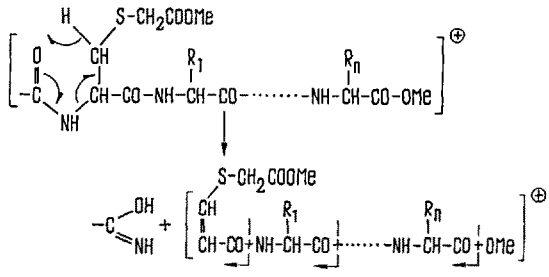


Molecular peaks are therefore absent in the mass spectra of S-β-aminoethylcysteine peptides, their mass spectra being very similar to those of the corresponding dehydroalanine peptides (see Figure 2). Since we have already shown[1,2] in studies of serine and threonine-containing peptides that the dehydroalanine derivatives undergo amino acid fragmentation, determination of the amino acid sequence of S-β-aminoethylcysteine peptides meets with no difficulties.

For the mass spectrometric determination of the amino acid sequence of cysteine peptides their S-carboxymethyl derivatives are most convenient. Moreover, blocking of the SH group by carboxymethylation is the most frequently used procedure in protein chemistry. Methyl esters of *N*-acyl-S-carboxymethylcysteine peptides are highly volatile and thermally stable, owing to which their mass spectra generally exhibit quite strong molecular ion peaks (see, for instance, the m/e 517 peak in Figure 3). The most characteristic fragmentation path for the molecular ion of such peptides is the amino acid type.

Peaks corresponding to the ions resulting from such fragmentation (see peaks at m/e 486, 458, 387, 330 and 302 in Figure 3) are usually quite strong and are accompanied by peaks lower by 73 and 106 m.u. (see peaks at m/e 444, 413, 411, 380, 352, 314, 281, 257, 224 and 196 in Figure 3). The latter are due to elimination, respectively, of a carbomethoxymethyl group or of methyl mercaptoacetate which occurs alongside the amino acid type of fragmentation. It is noteworthy that, in contrast to the cystine peptides, those with S-β-aminoethylcysteine and S-carboxymethylcysteine residues practically suffer no elimination of the side chain as a whole (i.e. no rupture of the C_α-C_β bond).

Still another process is characteristic of S-carboxymethylcysteine acylpeptide esters, namely, rupture of the



(7)

N-C_α-bond of the carboxymethylcysteine residue. This reaction, which we observed earlier with peptides containing aromatic and heterocyclic amino acids[1,2], is accompanied by migration of a hydrogen atom leading to ion (7) which undergoes further fragmentation by the amino acid mechanism. For instance in Figure 3 peak at m/e 346 corresponds to the ion of type (7), while its subsequent fragmentation gives the peaks at m/e 315, 287, 216, 188 and 159.

The findings described here show that the mass spectrometric method can be successfully employed for determining the amino acid sequence in cysteine-containing peptides formed in the partial hydrolysis of proteins.

Выводы. Показано, что масс-спектрометрический метод определения аминокислотной последовательности может быть с успехом применен к пептидам, содержащим остатки цистина, S-β-аминоэтилцистеина и S-карбоксиметилцистеина.

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Effect of Sodium Citrate on the Translocation of Bacterial DNA in *Solanum lycopersicum* esc.

It has been shown that foreign DNA can be taken up by plants[1–4]. After some depolymerization, though without modification of its primary and secondary structures, the DNA enters the cell nuclei where it appears to combine with the tomato DNA and replicate.

Since sodium citrate is known to precipitate or chelate bivalent cations, and because these ions, especially Ca^{++}, are known to be an important factor of cell permeability[5], plants were treated with sodium citrate in an attempt to increase the uptake of exogenous DNA.

Sodium citrate is known to inhibit DNAse I by its action on Mg^{++} ions[6] and so may also reduce the depolymerization of the foreign DNA translocated in the plants.

Plants of *Solanum lycopersicum* var. Tuckwood were used in the following experiments. Cut shoots (5–9 cm long) from adult plants with young leaves were placed in a solution of 0.15 M sodium citrate (tribasic) for 2 h. They were transferred to a solution of ^{3}H-DNA prepared from *Escherichia coli* (200 γ/ml) in dilute saline citrate solution for 6 h prior to placing in water for 48 h. As controls, cut shoots were placed in water instead of the citrate solution prior to similar feeding periods in the DNA solution and water.

A sample of internode (0.5 cm long) was removed from each plant, fixed for 3 h in Clark fixative and embedded in wax prior to sectioning for autoradiographic studies[7]. Some sections were used directly whilst others were (1) extracted with dilute acid to remove any labelled low-polymer polynucleotides, and (2) digested with DNAse I. Autoradiographs were exposed for 10 days.

After discarding the terminal portion of the shoot which had dipped in the feeding solutions, the DNA of the remainder of the material was extracted by a method already described[3]. This DNA was analyzed by centrifugation and chromatography on DEAE-cellulose columns[8].

A parallel study was made concerning the effects of citrate on the synthesis of endogenous DNA. Cut shoots were treated as described above except that the ^{3}H-DNA was replaced by a solution of ^{3}H-thymidine with a similar specific activity to that of the bacterial ^{3}H-DNA.

The bacterial DNA utilized in the experiments was extracted by the method of Marmur[9] from a thymine-less strain of *E. coli* (CR 34) which had been cultured on a medium containing ^{3}H-thymine.

As shown in Table I, shoots pretreated with citrate take up 3–4 times more exogenous DNA than the control plants. This result is confirmed by the autoradiographic study (Table II) where more labelled nuclei are found in each tissue from the citrate-treated shoots than the water-

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