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# Mass-Spectrometric Determination of the Amino Acid Sequences in Peptides Isolated from the Protein Silk Fibroin of *Bombyx mori*

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Several peptides were isolated from the protein silk fibroin of *Bombyx mori* by means of ion-exchange chromatography of a chymotryptic digest. The sequences of three of the peptides, Gly-Ala-Gly-Tyr, Gly-Val-Gly-Tyr and Gly-Ala-Gly-Ala-Gly-Ala-Gly-Tyr, were known from previous chemical work, but the sequence of the fourth, Gly-Ala-Gly-Val-Gly-Ala-Gly-Tyr, was previously only partially known. The necessary volatility for mass-spectrometric examination of the peptides was achieved by permethylation of the *N*-acetyl-peptide methyl ester derivatives. From the mass spectra it was possible to confirm the known sequences and to establish that of the partially known one. In one instance it was possible to deduce from the same mass spectrum the sequence of a main peptide component and that of a small amount of contaminating peptide. These results demonstrate for the first time the use of mass spectrometry in the determination of the amino acid sequences in peptides from a protein hydrolysate.

The first work on the determination of the amino acid sequences in peptides by mass spectrometry was done by Biemann, Gapp & Seibl (1959), who published a note describing a technique of reduction of the peptide to a polyamino alcohol followed by mass-spectrometric analysis. Later Biemann & Vetter (1960) combined the method with the g.l.c. separation of the polyamino alcohols and also added a further stage of reduction to the polyamines. The reduction was performed to increase the volatility of the peptides and, in the latter case, also to decrease the retention time on the chromatographic column. Heyns & Grützmacher (1963) used a direct-inlet system on their mass spectrometer for the introduction of some *N*-acetyl-peptides, and Weygand, Prox, Konig & Fessel (1963) studied the spectra of some *N*-trifluoroacetyl compounds. Since then the technique has been applied to some naturally occurring peptidolipids by Barber, Jollès, Vilkas & Lederer (1965) and Laneelle, Asselineau, Wolstenholme & Lederer (1965). Work has also been done on synthetic peptide derivatives in investigations to find more volatile derivatives suitable for mass spectrometry. Bricas *et al.* (1965) used *N*-acyl-peptide methyl esters, as also did Shemyakin *et al.* (1966).

Computer techniques have been used by Barber, Powers, Wallington & Wolstenholme (1966), Biemann, Cone, Webster & Arsenault (1966) and Senn & McLafferty (1966) for the interpretation of peptide mass spectra.

Thomas, Das, Gero & Lederer (1968) have shown that methylation of the peptide nitrogen gives enhanced volatility and a good spectrum was obtained from a naturally occurring pentadecapeptide.

Within the mass spectrometer the peptide derivatives are vaporized and then ionized by bombardment with a high-energy electron beam. The highly excited molecular ions (molecule minus one electron) can break down to give a number of characteristic fragment ions. Most of the fragments formed are due to cleavage across the peptide bonds.

The fragments resulting from cleavages of this kind are known as sequence ions, and the corresponding peaks in the mass spectrum, the sequence peaks, are usually the most intense in the spectrum. The amino acid sequence of a peptide can be deduced by fitting the mass difference between sequence-ion

peaks in the spectrum to the masses of the various amino acid residues. The spectrum is a plot of intensity versus mass/charge ratio ($m/e$). Since the charge is usually 1, for most peaks in the spectrum $m/e$ corresponds to mass units (mU).

A great deal of sequence information on a peptide can be obtained at low resolution, where the sequence peaks are measured to the nearest integral mass number. However, since the masses of the atoms are not simple whole numbers, different combinations that add up to the same nominal mass do in fact differ slightly in exact mass. For example, both N and $CH_2$ have a nominal mass of 14 units although their exact masses differ by 0·013 mU. This same mass difference can occur at higher masses; $C_{20}H_{36}N$ and $C_{21}H_{38}$, for instance, form a doublet at mass 290 also differing by 0·013 mU. If sufficiently high resolving power is used it is possible to separate such doublet peaks, and if the mass of any ion can be measured with sufficient accuracy its atomic composition can be determined.

For verification of a sequence, therefore, the instrument is tuned to operate at high resolution and a mass, accurate to 2 p.p.m., can usually be obtained, which enables the exact atomic composition of a given ion to be determined.

Accurate masses can be obtained at high resolution in two ways. By using the manual technique known as peak matching, the ratio of the ion-accelerating voltages necessary to bring a reference peak (of known accurate mass) and the sequence-ion peak to the same position on an oscilloscope display can be found. The ratio of the voltages gives the ratio of the masses, and hence the accurate mass of the sequence ion from which its atomic composition may be determined. The mass measurement of a peak in this way can take between 2 and 5 min., and it is unlikely that a small quantity of peptide (0·1–1 $\mu$mole) will last long enough to take more than a couple of measurements.

For very rapid accurate mass measurement of the complete spectrum, the output from the mass spectrometer (containing both reference and peptide peaks) can be fed into a computer via an analogue-to-digital converter. The computer is programmed to recognize the reference peaks and to extrapolate to find the accurate masses of the other peaks in the spectrum.

Up to the present time there has been no demonstration of an obvious application of the mass-spectrometric method of analysis, namely the determination of the primary structure of proteins. In the present paper we describe the sequence determination of several peptides obtained from a protein, the silk fibroin of the silkworm *Bombyx mori*.

Much progress has been made in the elucidation of the chemical and stereochemical structures of the silks and a review of this work has been given by Lucas & Rudall (1968). In the *Bombyx mori* fibroin molecule there seem to be three distinct regions or segments. Segment I is a polypeptide of 59 residues and consists for the most part of glycine residues alternating with alanine and serine in the remaining positions. Segment II consists mostly of glycine, alanine, valine and tyrosine, and is split by chymotrypsin into a variety of peptides in which two octapeptides and two tetrapeptides predominate. Segment III contains the polar and high-molecular-weight neutral amino acids and breaks down into a number of small peptides. We have used the mass spectrometer to examine several of the known and partially known sequences in segment II.

## EXPERIMENTAL

*Preparation of peptides.* The peptides were isolated from a chymotryptic digest of silk fibroin essentially by the method of Lucas, Shaw & Smith (1962). Slight modifications to their procedure were introduced by the use of the preparative column of the Phoenix automatic amino acid analyser.

An aqueous solution of silk fibroin (5 g.) was treated with chymotrypsin, and the soluble fraction Cs (consisting of peptides from segments II and III) was separated from the precipitate Cp (segment I), exactly as described by Lucas *et al.* (1962). A portion of fraction Cs, containing about 800 mg. of peptides in 100 ml., was applied to a column (150 cm. × 1·8 cm. diam. of Aminex-MS cation-exchange resin (8% cross-linking, 31–45 $\mu$m. in diameter) that had been equilibrated with 0·2 M-sodium formate buffer, pH 3·1. The column was run at a constant temperature of 30° by use of a thermostatically controlled water jacket. Buffer was pumped through the column at 100 ml./hr. A pH gradient was established by means of a mixing chamber (500 ml.) initially completely filled with the pH 3·1 buffer. A reservoir of 0·2 M-sodium acetate buffer, pH 5·1, was connected to the mixing chamber at the start of the elution, and this was changed to 0·2 M-NaOH after 1·8 l. had been eluted. The effluent from the bottom of the column was divided so that 10 ml./hr. was pumped to the analytical system of the analyser, and the remainder collected in a fraction collector (approx. 8 ml. fractions). The stream to the analyser was mixed with the standard 0·35 M-buffer, pH 5·28 (Spackman, Stein & Moore, 1958), pumped at 20 ml./hr., and then with ninhydrin reagent, pumped at 15 ml./hr. The colour was developed and recorded in the usual way (Spackman *et al.* 1958); an event-marking pen on the recorder was actuated by the fraction collector.

The chromatogram obtained is shown in Fig. 1. It differs in some respects from the chromatogram of the fractionation of fraction Cs obtained by Lucas *et al.* (1962), presumably because of the use of a different resin and temperature in the present experiment. The chromatograms are sufficiently alike for us to identify peak 10 of Fig. 1 with peak CsA (part of segment II) of the original fractionation. The fractions corresponding to peak 10 were combined, and a portion of the peptide mixture was adjusted to pH 2·5 and then fractionated on a column (110 cm. long × 1·7 cm. diam.) of Amberlite CG-50 by elution with the formate buffer, pH 3·1, at room temperature. The column

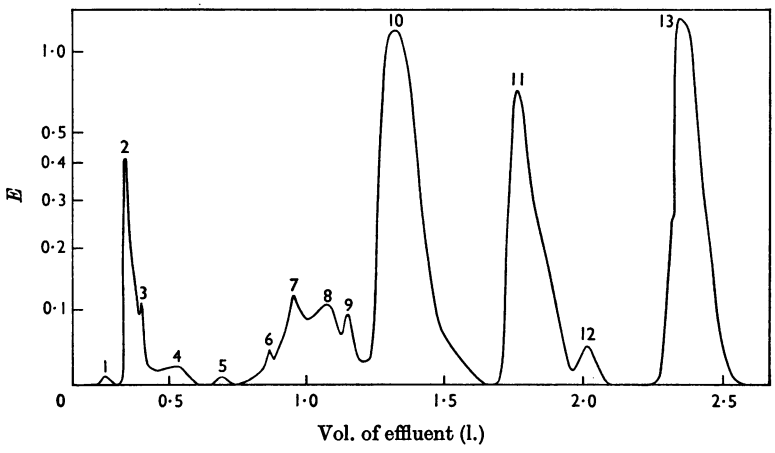


Fig. 1. Primary fractionation of fraction Cs (for details see the text).

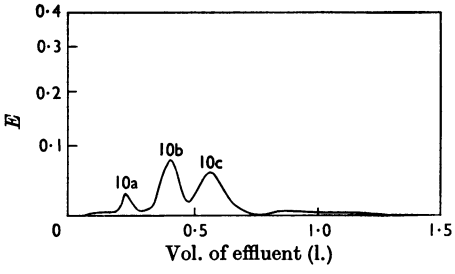


Fig. 2. Chromatography of fraction 10 from the primary fractionation.

effluent was divided and a portion analysed in the same way as in the primary fractionation described above.

In the chromatogram of the secondary fractionation (Fig. 2), the two main peaks 10b and 10c were expected to correspond to fractions $CsA_2$ and $CsA_3$ respectively described by Lucas *et al.* (1962). The fractions were desalted by the method of Dreze, Moore & Bigwood (1954) and portions were hydrolysed with 6M-HCl in evacuated sealed tubes at 110° for 24hr. The hydrolysates were analysed by the method of Spackman *et al.* (1958), with the following results: peptide 10b: $(Gly_{4\cdot0},Ala_{3\cdot2},Tyr_{1\cdot0})$; peptide 10c: $(Gly_{4\cdot1},Ala_{2\cdot1},Val_{1\cdot0},Tyr_{0\cdot6})$. Not more than 0·1 residue of other amino acids was found in the hydrolysates.

Because of the loss of tyrosine that occurred during the hydrolysis of peptide 10c, peptide 10b was hydrolysed in the presence of a small amount of phenol. From their chromatographic behaviour and composition, it is evident that peptide 10b can be identified with fraction $CsA_2$ and is the octapeptide:

Gly-Ala-Gly-Ala-Gly-Ala-Gly-Tyr

and peptide 10c corresponds to fraction $CsA_3$ and is the octapeptide:

Gly(Gly$_3$,Ala$_2$,Val)Tyr

Although the structure of the first octapeptide was completely established (Lucas *et al.* 1962) by a study of its terminal groups and the products of partial hydrolysis, these methods did not reveal the complete sequence of the second octapeptide.

In a further run, peak 11 (believed to correspond to a combination of peaks CsB and CsC described by Lucas *et al.* 1962) was separated and, on amino acid analysis, was found to contain: $(Gly_{1\cdot92},Ala_{0\cdot44},Val_{0\cdot71},Tyr_{1\cdot00})$ (together with much smaller amounts of other amino acids). For mass spectrometry peak 11 was not subjected to further fractionation. However, the sequence of fraction CsB was found to be Gly-Ala-Gly-Tyr (Lucas *et al.* 1962) and fraction CsC was determined as mainly the tetrapeptide Gly-Val-Gly-Tyr (see Lucas & Rudall, 1968).

*Preparation of permethylated* N*-acetyl-peptide methyl esters.* The isolated peptide fractions were evaporated to dryness by rotary evaporation. The residue was dissolved in 1% (v/v) acetic acid, transferred to weighed 2ml. glass ampoules, evaporated to dryness by means of a mechanical vacuum pump and weighed. The peptide methyl esters were obtained by dissolving the residue in excess of reagent, prepared by bubbling dry HCl through methanol (approx. 10$\mu$moles of peptide in 1ml. of reagent). The solutions were transferred to ampoules, which were then sealed and left overnight at room temperature. The ampoules were opened and the reagents taken off under vacuum.

*N*-Acetylation was achieved by dissolving the residue (about 10$\mu$moles) in excess (1ml.) of acetic acid–acetic anhydride (1:1, v/v). The solutions were transferred to fresh ampoules, which were sealed and left at room temperature for 4hr. The reagents were taken off under vacuum in the presence of NaOH.

The resulting *N*-acetyl-peptide methyl esters were subjected to the permethylation technique described by Das, Gero & Lederer (1967). Approx. 5mg. of residue was dissolved in 1ml. of anhydrous dimethylformamide and treated with 0·6ml. of methyl iodide in the presence of 0·3g. of $Ag_2O$ (from BDH Chemicals Ltd., Poole, Dorset) for 3 days with continuous stirring.

This procedure has since been modified to decrease the

reaction time (see Thomas *et al.* 1968). The mixture was filtered and chloroform (about 20ml.) added to the filtrate until no more solid separated. The solid was removed by washing with 5% KCN solution and separating the aqueous layer. The chloroform layer was washed repeatedly with water, dried over anhydrous $Na_2SO_4$ and evaporated to dryness, yielding the permethylated derivatives.

*Mass spectrometry.* All mass spectra were recorded on an AEI MS902 double-focusing instrument. In each case the peptide was admitted to the ion source on a direct-insertion probe. A suitable rate of evaporation was obtained with an ion-chamber temperature of 220°. For each compound a low-resolution (about 1000) spectrum was recorded. Accurate mass measurements were then performed by a small computer (PDP-8; Digital Equipment Corporation) connected on-line to the mass spectrometer. For these measurements the spectra were recorded at a resolving power of 10000 and a scan time of 35sec./decade in mass. The reference compound was perfluorokerosene. Owing to the lack of suitable reference peaks in the perfluorokerosene spectrum, the highest mass peaks in the peptide spectra, e.g. *m/e*832, were measured by the peak-matching technique. Wide ratios were employed (see Fales, Binks, Elliott & Freemann, 1966) so that lower-mass reference peaks could be used.

## RESULTS

Fig. 3 shows a line drawing of the mass spectrum of the partially known octapeptide from fraction 10c. The very-low-intensity peak at *m/e*832 is the expected molecular ion from the octapeptide having the amino acid analysis ($Gly_4$,$Ala_2$,Val,Tyr). Accurate mass measurement (832·4705) of this ion confirmed the molecular formula $C_{40}H_{64}N_8O_{11}$ (832·4695).

As was explained in the introduction, the sequence of a peptide can be deduced by observing the peaks that are due to the charged peptide portions remaining on each sequential loss of an amino acid residue from the *C*-terminus.

The peak at *m/e* 610 ($C_{28}H_{48}N_7O_8$) is then due to the peptide portion remaining after the loss of the *C*-terminal *NO*-dimethyltyrosine methyl ester ($Me_2$Tyr-OMe unit). As confirmatory evidence should be noted also the intense peaks at *m/e*121 ($C_8H_9O$) and *m/e*192 ($C_{11}H_{12}O_3$), both derived from the methylated tyrosine unit. The next relatively intense peak lower in mass is *m/e*539. This is due to $C_{25}H_{43}N_6O_7$ and is the result of simple cleavage of the next peptide bond from the *C*-terminus; the amino acid unit lost in the fragmentation is MeGly (71mU, $C_3H_5NO$). The next peak of significant intensity is at *m/e*454 ($C_{21}H_{36}N_5O_6$) corresponding to the loss of a MeAla unit. Further loss of 71mU, i.e. a MeGly unit, gives *m/e*383, and then a MeVal unit (113mU) is lost to give *m/e*270 ($C_{12}H_{20}N_3O_4$). The sequence is thus so far quite unambiguously identified as:

-MeVal-MeGly-MeAla-MeGly-$Me_2$Tyr-OMe

The next most intense peak in the spectrum is *m/e*199, due to loss of a MeGly unit from *m/e*270. The intense peak at *m/e*114 is due to the terminal *N*-acetyl-MeGly unit and represents 85mU, i.e. a MeAla unit from *m/e*199. The complete sequence of the methylated peptide is thus shown to be:

Acetyl-MeGly-MeAla-MeGly-MeVal-MeGly-MeAla-MeGly-$Me_2$Tyr-OMe

This was thought to be the most likely sequence of the peptide by Lucas *et al.* (1962), but the chemical evidence did not allow an unambiguous choice to be made from two possible sequences.

There are other intense peaks in the mass spectrum that are not necessary for sequence determination. An example of this is the peak at *m/e*634, which is due to an ion that has the atomic composition $C_{31}H_{50}N_6O_8$ and arises presumably by loss of the acetyl-MeGly-MeAla-H unit.

A further check on this sequence determination was made by comparing the spectrum with that of the methylated peptide of known sequence from fraction 10b. A line drawing of the spectrum of this latter compound is shown in Fig. 4. It should be noted that the peaks up to and including *m/e*270 occur at the same masses as for the unknown peptide. After *m/e*270, however, the next intense peak is at *m/e*355 rather than *m/e*383. This reflects the presence, at this point in the sequence, of a MeAla unit instead of the MeVal unit in the peptide whose sequence was only partially known. It should be noted also, however, that *m/e*383 is also present, its intensity being approx. 50% that of *m/e*355. This same pattern of a peak 28mU higher in mass occurs also at *m/e*426, *m/e*511, *m/e*582 and *m/e*606. The very low intensity of the molecular-ion peak at *m/e*804 prevents a reliable measure of the expected peak of even lower intensity at *m/e*832. However, this spectrum indicates clearly the difference between this known octapeptide derivative and the partially known one as the presence of a MeAla unit instead of a MeVal unit as the fourth amino acid unit from the *N*-terminus. A simple comparison of the mass spectra provides this result as well as the evidence that the peptide of known structure was contaminated with approx. 30% of the valine-containing peptide.

The amino acid analysis of peptide 10b did not reveal any contamination by the valine-containing peptide. The sample used for mass spectrometry was prepared in a different experiment from that used for chemical analysis, and it is evident that the resolution of the octapeptides in this experiment was not complete.

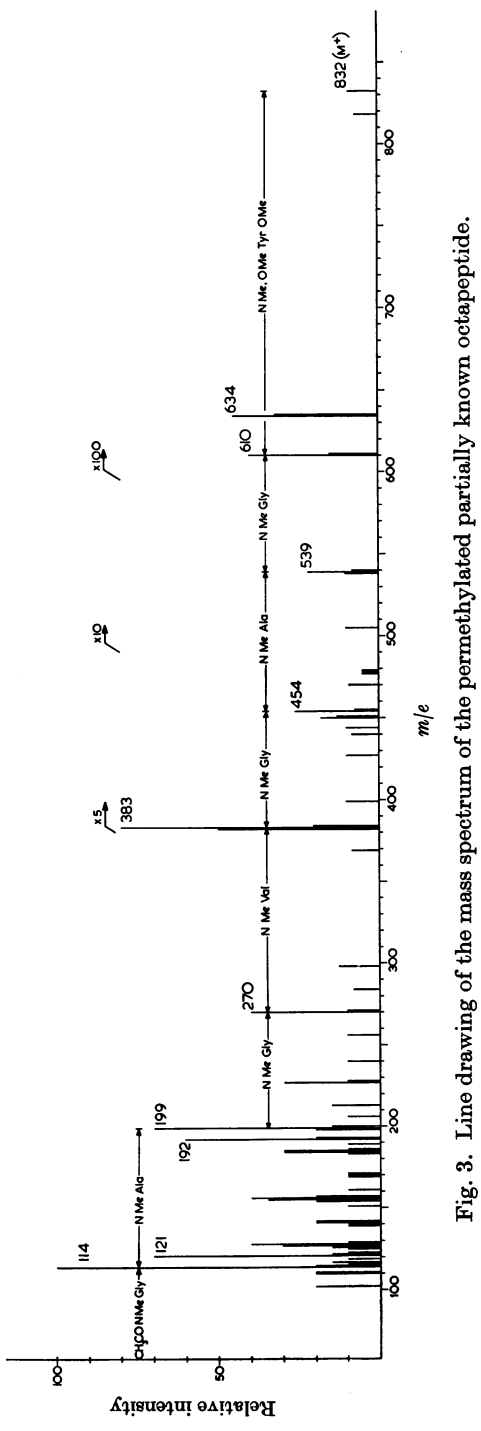


Fig. 3. Line drawing of the mass spectrum of the permethylated partially known octapeptide.

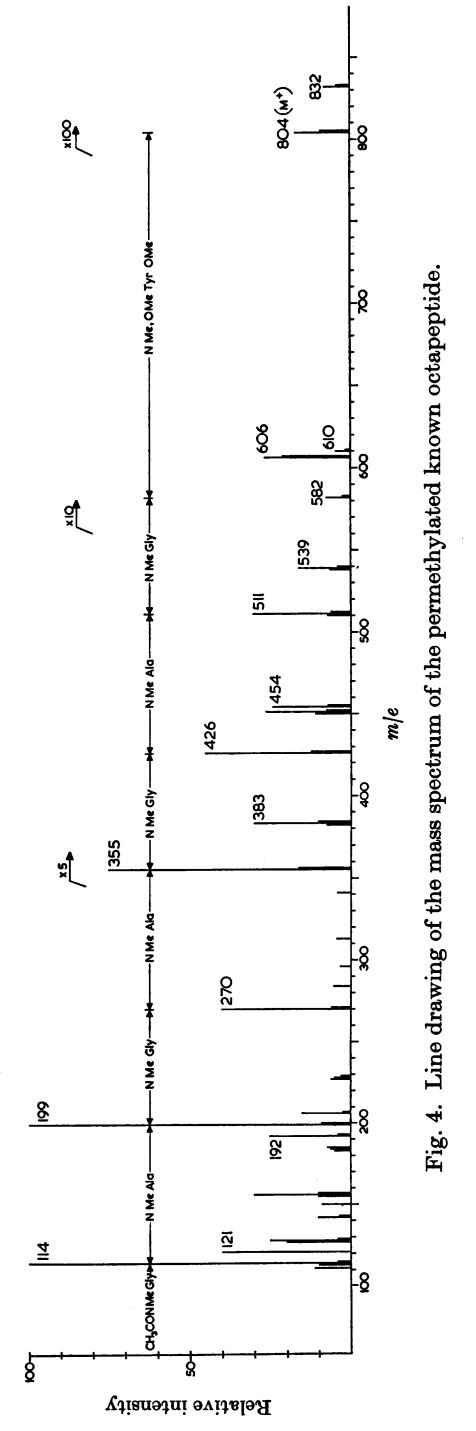


Fig. 4. Line drawing of the mass spectrum of the permethylated known octapeptide.

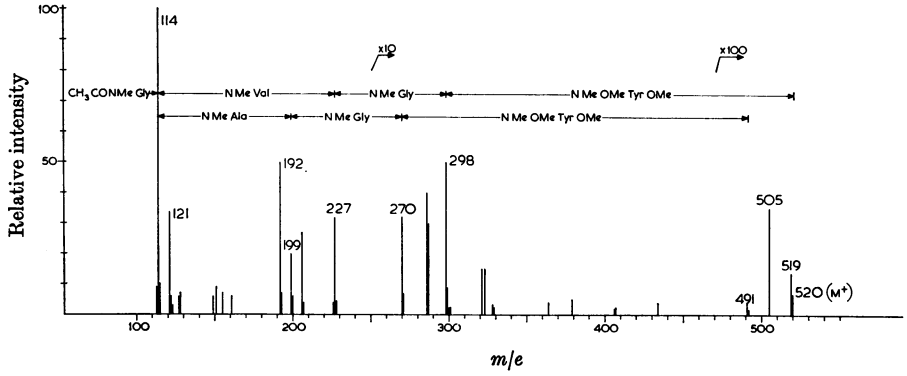


Fig. 5. Line drawing of the mass spectrum of the permethylated tetrapeptide.

Fig. 5 shows the line drawing of the spectrum of the permethylated tetrapeptide comprising peak II. This shows very clearly the presence of two different compounds. The base peak is *m/e* 114 and the computer print-out of the complete high-resolution spectrum shows this to be due to $C_5H_8NO_2$. This corresponds to acetyl-MeGly. The peak at *m/e* 121 corresponds to the *p*-methoxybenzyl group of the tyrosine side chain, and the intense peak at *m/e* 192 is also derived from the *C*-terminal tyrosine unit. The peak at *m/e* 227 ($C_{11}H_{19}N_2O_3$) is due to acetyl-MeGly-MeVal, but the smaller peak at *m/e* 199 should also be noted. The complete high-resolution scan showed this to be a doublet. The major component is $C_9H_{15}N_2O_3$, which corresponds to acetyl-MeGly-MeAla. The other component is $C_{10}H_{19}N_2O_2$, due to loss of CO from *m/e* 227. This is a familiar fragmentation in linear peptides and is confirmed in this case by a metastable peak at *m/e* 174·5. Peaks are then observed at *m/e* 270 and *m/e* 298. These are due to acetyl-MeGly-MeAla-MeGly and acetyl-MeGly-MeVal-MeGly respectively. The molecular ions of the two peptides occur at *m/e* 520 and *m/e* 492. In each case a more intense peak at $M-1$ (molecular ion minus one hydrogen atom) is observed. This permethylated peptide is thus shown to be a mixture of the two tetrapeptides acetyl-MeGly-MeVal-MeGly-Me$_2$Tyr-OMe and acetyl-MeGly-MeAla-MeGly-Me$_2$Tyr-OMe. The ratio of the concentrations of the two is approx. 3:1, the valine-containing compound being the major constituent. The results of the accurate mass measurements are in agreement with the above interpretation.

## DISCUSSION

One of the factors that, it has been thought, could possibly hinder the peptide sequence determination by mass spectrometry is the presence of impurities. This is of particular importance in peptides separated from protein hydrolysates. However, in the example of the octapeptide of partially known sequence described above, fractionation of the impurity–peptide mixture occurred on the direct-insertion probe, thereby affecting a partial separation. Four complete high-resolution spectra were recorded consecutively and the results showed clearly the presence of hydrocarbon impurity in scan 1 and the relative lack of it and greater intensity of the peptide peaks in scan 4. This was due to the different volatilities of the peptide and impurity, the latter being the more volatile in this case. Such a difference in volatilities may not always occur and it is possible that the peptide might have approximately the same volatility as the impurity. This would lead to masking of the spectrum, and this problem would have to be overcome either by further purification of the peptide, or by a complete high-resolution scan. Since the complete high-resolution spectrum provides the atomic composition of each peak in the spectrum, impurities can readily be recognized even when they are mixed with the peptide. This may well not be the case if only limited high-resolution results are obtained, since the mixing may occur in such a way that the peptide spectrum is largely masked under low-resolution conditions. One would not therefore know which peaks to choose for a limited high-resolution study. Fig. 6 shows the computer print-outs for the mass range *m/e* 393 to *m/e* 355 for scans 1 and 4. The hydrocarbon-impurity peaks at *m/e* 391, 385, 379, 378 and 375 on the print-out for scan 1 should be noted. These are absent from scan 4, although the peptide peaks, e.g. *m/e* 383, are of greater intensity on this scan. The peaks due to non-peptide impurity are thus readily identified and differentiated from the peptide spectrum.

REPEAT SCANS ON OCTAPEPTIDE

| CALCULATED MASS | ERR | C12/13 | H | N | O | MEASURED MASS | NO. PTS | INTENSITY |
|---|---|---|---|---|---|---|---|---|
| SCAN 1: | | | | | | | | |
| REFERENCE | | | | | | 392.9761 | 12 | +++++++ |
| 391.4303 | -3.35 | 28/0 | 55 | 0 | 0 | 391.4269 | 7 | +++++ |
| 385.3833 | 2.13 | 28/0 | 49 | 0 | 0 | 385.3855 | 5 | ++++ |
| 383.2320 | -2.80 | 21/0 | 29 | 5 | 2 | 383.2291 | 8 | ++++++ |
| 383.2293 | - .12 | 18/0 | 31 | 4 | 5 | | | |
| 383.2307 | -1.46 | 20/0 | 33 | 1 | 6 | | | |
| 382.2170 | 3.05 | 27/0 | 28 | 1 | 1 | 382.2200 | 8 | ++++++ |
| 382.2215 | -1.46 | 18/0 | 30 | 4 | 5 | | | |
| 382.2228 | -2.80 | 20/0 | 32 | 1 | 6 | | | |
| 382.2175 | 2.56 | 13/0 | 30 | 6 | 7 | | | |
| REFERENCE | | | | | | 380.9760 | 9 | +++++++ |
| 379.4304 | - .30 | 27/0 | 55 | 0 | 0 | 379.4301 | 6 | +++++ |
| 378.4224 | -1.46 | 27/0 | 54 | 0 | 0 | 378.4210 | 6 | ++++ |
| 375.3989 | 3.29 | 27/0 | 51 | 0 | 0 | 375.4023 | 7 | +++++ |
| REFERENCE | | | | | | 368.9760 | 10 | ++++++ |
| 368.3514 | -1.95 | 21/0 | 44 | 4 | 1 | 368.3494 | 9 | +++++ |
| 368.3528 | -3.29 | 23/0 | 46 | 1 | 2 | | | |
| 368.2126 | - .36 | 25/0 | 26 | 3 | 0 | 368.2122 | 5 | ++++ |
| 368.2140 | -1.64 | 27/0 | 28 | 0 | 1 | | | |
| 368.2086 | 3.66 | 20/0 | 26 | 5 | 2 | | | |
| 368.2099 | 2.38 | 22/0 | 28 | 2 | 3 | | | |
| 368.2144 | -2.19 | 13/0 | 30 | 5 | 7 | | | |
| SCAN 4: | | | | | | | | |
| REFERENCE | | | | | | 392.9761 | 13 | +++++++ |
| 384.2313 | 1.95 | 25/0 | 28 | 4 | 0 | 384.2332 | 8 | +++++ |
| 384.2326 | .54 | 27/0 | 30 | 1 | 1 | | | |
| 384.2300 | 3.29 | 24/0 | 32 | 0 | 4 | | | |
| 384.2331 | .12 | 13/0 | 32 | 6 | 7 | | | |
| 383.2320 | -2.31 | 21/0 | 29 | 5 | 2 | 383.2297 | 13 | +++++++ |
| 383.2334 | -3.66 | 23/0 | 31 | 2 | 3 | | | |
| 383.2293 | .36 | 18/0 | 31 | 4 | 5 | | | |
| 383.2307 | - .97 | 20/0 | 33 | 1 | 6 | | | |
| REFERENCE | | | | | | 380.9760 | 13 | +++++++ |
| 369.2165 | -1.83 | 20/0 | 27 | 5 | 2 | 369.2146 | 11 | ++++++ |
| 369.2176 | -3.11 | 22/0 | 29 | 2 | 3 | | | |
| 369.2137 | .91 | 17/0 | 29 | 4 | 5 | | | |
| 369.2151 | - .48 | 19/0 | 31 | 1 | 6 | | | |
| MISSING REFERENCE | | | | | | | | |
| 355.2048 | -1.83 | 24/0 | 25 | 3 | 0 | 355.2030 | 5 | +++++ |
| 355.2061 | -3.11 | 26/0 | 27 | 0 | 1 | | | |
| 355.2008 | 2.19 | 19/0 | 25 | 5 | 2 | | | |
| 355.2020 | .91 | 21/0 | 27 | 2 | 3 | | | |
| REFERENCE | | | | | | 354.9792 | 12 | ++++++ |

Fig. 6. Parts of the computer print-outs from runs 1 (above) and 4 (below) of the complete high-resolution spectra of the permethylated partially known octapeptide. It should be noted that the computer gives all possible atomic compositions that lie within $\pm 10$ p.p.m. of the measured mass of a particular peak. Column 1 gives the calculated mass for the listed atomic composition; column 2 gives the difference between the calculated mass and that measured by the computer; column 3 give the possible atomic composition (C, $^{13}C$, H, N and O are included in this case); column 4 gives the mass measured by the mass spectrometer–computer system; column 5 gives the number of digital samples in the peak; column 6 gives a measure of the peak intensity on a logarithmic scale.

Acetylated octapeptide methyl esters that had not been permethylated were also investigated. It was found that these compounds were not sufficiently volatile to give a spectrum. It was also noted that the presence of impurities apparently did not interfere with the permethylation of the peptides.

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