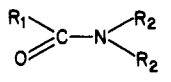
and a comparison of the data in at least these two solvents should be justified. The 50% difference in $\Delta H^{\ddagger}$ far exceeds the experimental error and is likely to reflect a genuine difference in the solvent effect. The most likely reason for the unusually high barrier to rotation of I in the liquid-crystal solution may be due to the ordering forces of the solvent rather than electronic forces. In its transition state, the plane of the dimethylamino group is perpendicular to that of the pyrimidine ring. The solute molecule in this conformation would disturb the ordering of the solvent molecules in its vicinity more than the ground state would. Therefore, the strong ordering forces of the liquid-crystal solvent would not favor the transition state and would cause the rotational barrier of I to increase. Although the explanation is a conjecture without sophisticated statistical calculations as a proof, it seems to be a reasonable one from the point of view that in liquid-crystal solutions planar molecules usually have larger ordering factors than globular molecules. It is for this reason that we chose to start our investigation on hindered rotation in liquid crystals using compound I rather than the classical examples of amides. If R_1



III

in III is small (e.g., H or CH_3), the transition state in which the R_2—N—R_2 plane is perpendicular to the R_1—C=O plane may not perturb the ordering of the liquid-crystal solvent much more than the planar ground state does. On the other hand, if R_1 is large (e.g., phenyl), the ground state may be quite bulky and even non-planar and would differ little from the transition state in perturbing the solvent ordering.

In the discussion presented above, we have treated the system as having a single transition state in which the dimethylamino group is perpendicular to the ring in I. Actually, in any condensed medium, the existence of several transition states with the solute interacting differently with the solvent should be considered. The values of $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, and $\Delta G^{\ddagger}$ are ensemble averages.[30] In other words, the neighboring packing effect of the liquids[31] would affect the activation parameters in addition to the polarity and hydrogen-bonding character of the solvent. This effect is quite different in liquid crystals and is highly anistropic. The details of the packing effect of liquid crystals on molecular conformations and dynamics remain to be investigated. Our observation of an unusually large value of $\Delta H^{\ddagger}$ for the hindered rotation of I in liquid-crystal solutions is an interesting experimental result, and similar studies on other systems should be pursued.

In summary, we have demonstrated for the first time that natural abundance carbon-13 NMR can be used to study dynamic processes in liquid-crystal solutions. It was observed that $\Delta H^{\ddagger}$ of the hindered rotation of the dimethylamino group in 4-(dimethylamino)pyrimidine in the liquid-crystal solvent ZLI 2142 is 50% higher than that in CD_2Cl_2. The most likely reason for this is that the non-planar transition state perturbs the ordering of the liquid-crystal solvent and becomes less favorable so that the barrier to rotation increases over that in CD_2Cl_2.

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On-Line Peptide Sequencing by Enzymatic Hydrolysis, High Performance Liquid Chromatography, and Thermospray Mass Spectrometry

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Abstract: On-line procedures for peptide sequencing using columns containing immobilized enzymes, carboxypeptidase Y (CPY) and trypsin, and an LC column, coupled to a thermospray mass spectrometer, are described. After on-line hydrolysis by immobilized CPY, both the amino acids, sequentially cleaved from the C terminus of the peptides, and the residual peptides are carried into the thermospray ion source by a continuous buffer flow and detected as molecular ion species. Use of both positive and negative ion modes gives additional flexibility to detect these molecules. For large peptides, on-line proteolytic hydrolysis into peptide fragments using immobilized trypsin can be employed along with separation by an LC column before hydrolysis by immobilized CPY. Immobilized trypsin alone can be used with LC separation for peptide mapping. These procedures provide rapid and convenient sequencing methods with high sensitivity. Total experimental times are usually less than 30 s for small peptides and detection limits extend to the low picomolar range.

Introduction

"Soft ionization" mass spectrometric techniques for sequencing peptides and for detection of biological peptides have found increasing use recently. Unlike conventional mass spectrometry, these techniques permit the analysis of thermally labile and/or nonvolatile molecules, usually without derivatization. Of the several "soft ionization" techniques, plasma desorption,[1] field desorption (FD),[2-5] fast atom bombardment (FAB),[6-10] and thermospray liquid chromatography/mass spectrometry (LC/

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MS)[11-14] have been the most successful for analyzing underivatized peptides. As an on-line operation, thermospray LC/MS has been found to be of particular value, offering a fast and convenient method for analyzing a variety of compounds and mixtures.

The thermospray technique was originally developed as a vaporization method to introduce the samples eluting from an LC into a chemical ionization (CI) mass spectrometer.[15] When the mobile phase contains a significant concentration of electrolytes (ca. 10^{-4} to 1 M), it was found that the thermospray process itself also can serve as an ionization method without an external ionizing source.[11-13] Spectra obtained this way show abundant molecular ion species with few fragments and low background. Since thermospray ionization includes vaporization of ions preformed in solution as well as gas-phase proton-transfer reactions,[16] this system is particularly well suited to peptide samples. Furthermore, the thermospray ion source can handle flow rates of aqueous buffers up to 2 mL/min, allowing practical on-line HPLC separation of a peptide mixture before detection.

Studies of sequencing peptides employing FD and FAB mass spectrometry have been carried out either by direct fragmentation[17,18] or in conjunction with Edman degradation[19,20] or enzyme hydrolysis.[21-24] Despite successful results reported for many peptides, the extensive off-line sample preparation required for FD makes efficient sample handling difficult. In the cases where FAB is applied to sequencing by direct fragmentation, the lack of the complete fragmentation of peptide bond does not permit total sequence elucidation. When enzymatic hydrolysis is used together with FAB, both enzymatically and mass spectrometrically generated fragments often complicate reliable sequence determination. Recently, thermospray LC/MS has been applied to peptide sequencing using on-line enzymatic hydrolysis by immobilized carboxypeptidase Y (CPY). CPY cleaves amino acids from the C terminus of peptides in a stepwise manner. C-terminal sequences of several amino acid residues from peptides and proteins have been successfully determined by this method.[25,26]

In the present study, rapid and efficient sequencing methods which can be applied to most peptides are reported. This work employs on-line digestion of peptides using two different enzyme columns (immobilized CPY and immobilized trypsin, on-line separation with a HPLC column, and on-line detection with a thermospray mass spectrometer. The two enzyme columns can be used separately, with or without LC separation, or in conjunction with each other. In the latter case, tryptic fragments generated in the trypsin column are separated with an LC column prior to digestion by immobilized CPY. The enzyme hydrolysates are carried into the thermospray ion source by a continuous flow of an aqueous buffer and detected mainly as molecular ion species. Both positive and negative ion modes can be employed for their detection. The resultant molecular ions including C-terminal amino acids, residual N-terminal peptides, and tryptic fragments give a variety of information about the sequences of their parent peptides. The total amount of sample needed is usually in the low nanomolar level, although it varies depending on peptides. For some favorable cases, the detection limit is in the low picomolar range.

Experimental Section

The thermospray LC/MS system uses a slightly modified Biospect mass spectrometer equipped for CI operation. Our current version of the thermospray interface consists of a stainless steel capillary vaporizer (0.15 mm i.d. × 1.5 mm o.d. × 30 cm long) which is heated by passing an electrical current through the capillary. Heat input is regulated by feedback control on the power supply using a thermocouple to maintain a given temperature at the vaporizer.[27] Since the low heat capacity of capillary tube also allows fast heat transfer between liquid stream and heated vaporizer, this provides an efficient way to compensate the flow fluctuation introduced by the LC pump. Conditions were optimized by changing the vaporizer temperature along with source temperatures.

All solutions were delivered by a Spectra-Physic Model SP 8700 pump. Sample solutions were injected with a Rheodyne 7010 injector with a 20-μL sample loop. All the data were acquired with a Finnigan Incos Data System.

Carboxypeptidase Y (CPY) (EC 3.4.12.1), generously provided by Dr. J. T. Johansen, was immobilized on long-chain alkylamine controlld pore glass beads (LCAA/CPG) with 125–177-μ bead size and 500-Å nominal pore diameter using glutaraldehyde linkage as described earlier.[24,25] Unreacted functional groups were covered with 0.5 M glycine. The immobilization method was considerably improved by reducing unstable imine bonds (–N=CH–) to single bonds (–NH—CH_2–) by using sodium cyanoborohydride[28] at pH 7.0. With this method stable enzyme activity was maintained over 6 months, even when samples were used under high pressure (up to 3000 psi).

Trypsin, obtained from Sigma Chemical Co. (St. Louis, MO) and purified by ion-exchange chromatography to remove the chymotryptic contamination,[29] was immobilized on carbonyl diimidazole functionalized controlled pore glass beads (CDI/CPG) purchased from Pierce Chemical Co. (Rockford, IL) following the manufacturer's specification.[30] The bead size and nominal pore diameter were 125–177 μ and 500 Å, respectively. Enzyme columns were packed by a slurry method under pressures up to 4000 psi in stainless steel tubes of 2.1 mm i.d. The column length was varied in the range of 4–20 cm depending on the degree of enzyme activity and sample dispersion desired. A synthetic pentapeptide Phe-Leu-Glu-Glu-Ile, dynorphin fragment 1–13, and α-MSH were purchased from Sigma Chemical Co., and were used without further purification. All peptide samples were dissolved with deionized water to 10^{-4} M. Ammonium acetate buffer was used to deliver the sample solutions to the thermospray ion source. For peptide separation, an Ultrasil-NH_2 column (10 μ, 4.6 mm × 20 cm) manufactured by Altex Scientific Operations (Berkeley, CA) was used.

Results and Discussion

(1) On-Line Hydrolysis with Immobilized CPY/Thermospray MS. Our original approach for this study employed on-line CPY digestion followed by quantitation of the sequentially released C-terminal amino acids with the thermospray mass spectrometer.[25,26] A major improvement has been achieved by monitoring both the released C-terminal amino acids and the residual peptides

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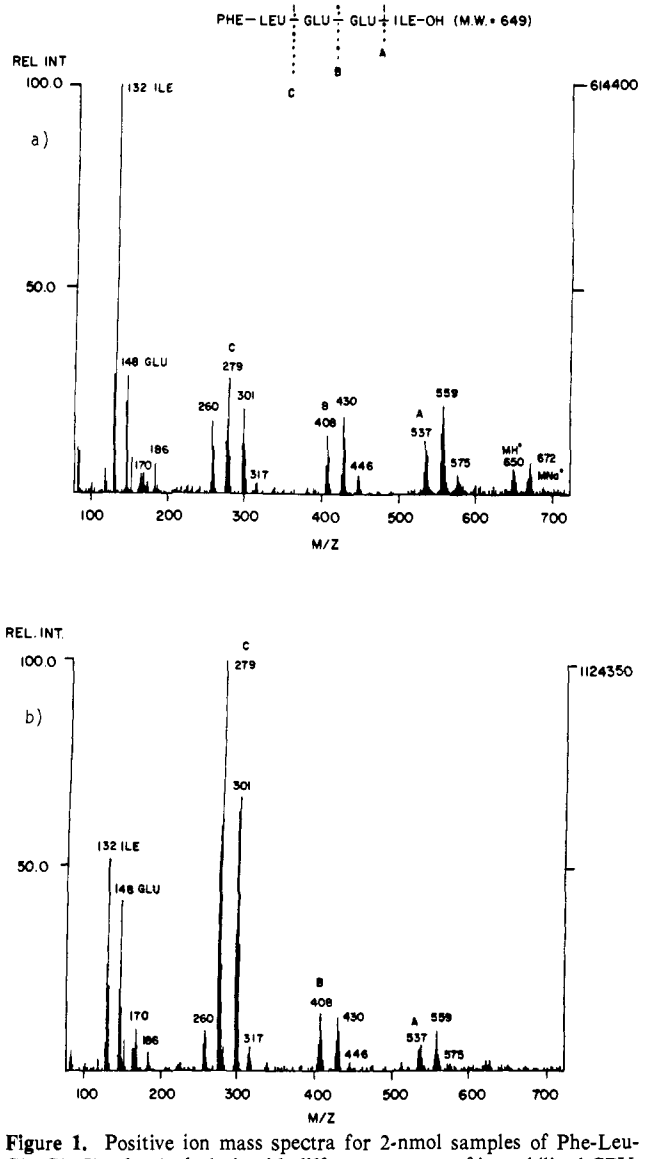

Figure 1. Positive ion mass spectra for 2-nmol samples of Phe-Leu-Glu-Glu-Ile after hydrolysis with different amounts of immobilized CPY: (a) 2.5×10^{-8} mol; (b) 1.4×10^{-7} mol. Further conditions are given in the text. A, B, and C in the spectra represent the protonated molecular ions for the residual peptides produced by hydrolysis from the C terminus at A, B, and C, respectively.

(remaining after successive cleavage of these amino acids). This permits unambiguous sequence determination, allowing the correct location of repeated amino acids in the peptide chain. This latter information is often difficult to deduce strictly by quantitating the hydrolyzed amino acids. The quantitation method can still be employed to provide the complementary information, by using unit mass resolution to differentiate amino acids with proximate molecular weights, for instance, Glu and Gln. Since thermospray ionization produces little information about fragmentation patterns, Leu and Ile are not distinguishable.

Samples of 2 nmol of a synthetic peptide, Phe-Leu-Glu-Glu-Ile (MW 649), were injected through columns containing different amounts of immobilized CPY. The C-terminal amino acids and residual peptides produced by sequential hydrolysis in the CPY column were carried into the thermospray ion source by 0.1 M ammonium acetate buffer at pH 5.5. Figures 1a and 1b repesent the positive ion spectra obtained at room temperature by using columns containing 2.5×10^{-8} and 1.4×10^{-7} mol of CPY, respectively. The flow rate was 1 mL/min for both cases. Both spectra show the protonted molecular ions of the amino acids, Ile and Glu, and the residual peptides along with their alkali ion adducts. The sodium and potassium adducts appear 22 and 38 mass units higher than the protonated molecules, confirming that these are singly charged species.[14] The residual peptide peaks at m/z 537, 408, and 279 allow a correct determination of the sequence, including the repeated glutamic acid residues in the chain.

Figure 1a shows an additional interesting phenomenon. For the intact molecule and the residual peptides Phe-Leu-Glu and Phe-Leu-Lglu-Glu, the sodium adducts of the molecules appear with higher intensity than the protonated molecular ions. However, the residual peptide Phe-Leu gives higher protonated molecular ion than the sodium adduct peak. The absolute intensities of the sodium adducts depend on the amount of sodium contaminant in the sample, the buffer, or the ion source at any given time. In all cases sodium ions are present as trace contaminants. Nevertheless, it has been consistently observed that molecules containing acidic residues give higher relative intensities of sodium adduct peaks than do molecules without acidic residues. This peak profile therefore provides additional evidence of correct sequence location of the acidic amino acids.

With a larger amount of enzyme the extent of hydrolysis was increased, and the molecular ion species for the intact peptide are no longer visible (Figure 1b). In their place, a major increase in the molecular ion species for the dipeptide Phe-Leu is seen, indicating the accumulation of this species. CPY hydrolyzes unprotected dipeptides very slowly. The peak at m/z 260 is an unknown impurity in the original sample (see also Figure 2). Although controlling the extent of hydrolysis does not provide new information in the case of this small peptide, it is important for large peptides where it can permit penetration much further into the amino acid sequence. Besides changing the amount of enzyme in the column, the extent of hydrolysis can also be controlled by changing column temperatures, flow rates, and the pH of the buffer solution.

Negative ion spectra can also provide useful sequence information. With ammonium acetate buffer the negative ion detection mode gives virtually no background except m/z 119, which is $CH_3COOH{\cdot}CH_3COO^-$, and the corresponding sodium adduct $CH_3COONa{\cdot}CH_3COO^-$ at m/z 141. Figure 2a shows the molecular ion $(M - H)^-$ of the starting molecule, without digestion by CPY. Other than an unknown peak at m/z 97, and the m/z 141 from the background, the peptide sample mainly produced $(M - H)^-$ at m/z 648. After hydrolysis with immobilized CPY (same conditions as for Figure 1a), deprotonated molecular ions of C-terminal amino acids and residual peptides were detected as shown in Figure 2b. In the negative ion mode, alkali ion attachment is not usual, thus producing simpler spectra. Since it is considered that molecular ion species of large peptides are produced mainly by vaporization of ions preformed in solution whereas those of small peptides could also be produced by ion–molecule reaction in the gas phase, the charge status of peptide samples is quite important for their detection. The use of both positive and negative ion modes permits more flexibility with samples regardless of their charge status. Complementary information about residual peptides which cannot be detected in one mode can be obtained by using the other mode.

For relatively small peptides (with MW below 1500) sequencing with immobilized CPY generates sufficient information to allow almost complete sequence determination with great speed. The largest peptide which has been sequenced successfully by this technique is the tetradecapeptide renin substrate (MW 1758), for which 11 amino acid residues were unambiguously determined using three different hydrolysis conditions.[31] The sample throughput is usually less than 30 s, although this depends on enzyme column dimensions and flow rates.

(2) On-Line Tryptic Digestion/HPLC Separation/Thermospray MS. We have already reported on the application of the thermospray interface to detect the nonderivatized tryptic peptides.[14] In the present work this has been expanded to develop a fast and reliable technique for peptide mapping, using on-line digestion by immobilized trypsin followed by LC fractionation. The sep-

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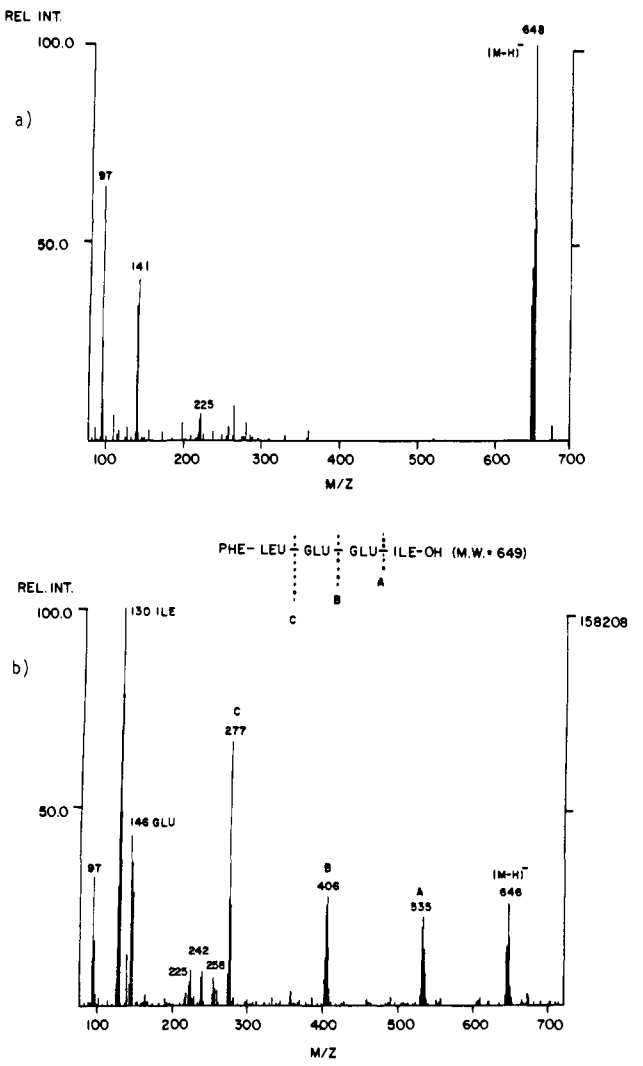

Figure 2. Negative ion mass spectra for 2-nmol samples of Phe-Leu-Glu-Glu-Ile: (2a) obtained without hydrolysis; (b) result for hydrolysis by immobilized CPY as in Figure 1a. A, B, and C in (b) represent the deprotonated molecules of the residual peptides produced by hydrolysis at A, B, and C, respectively.

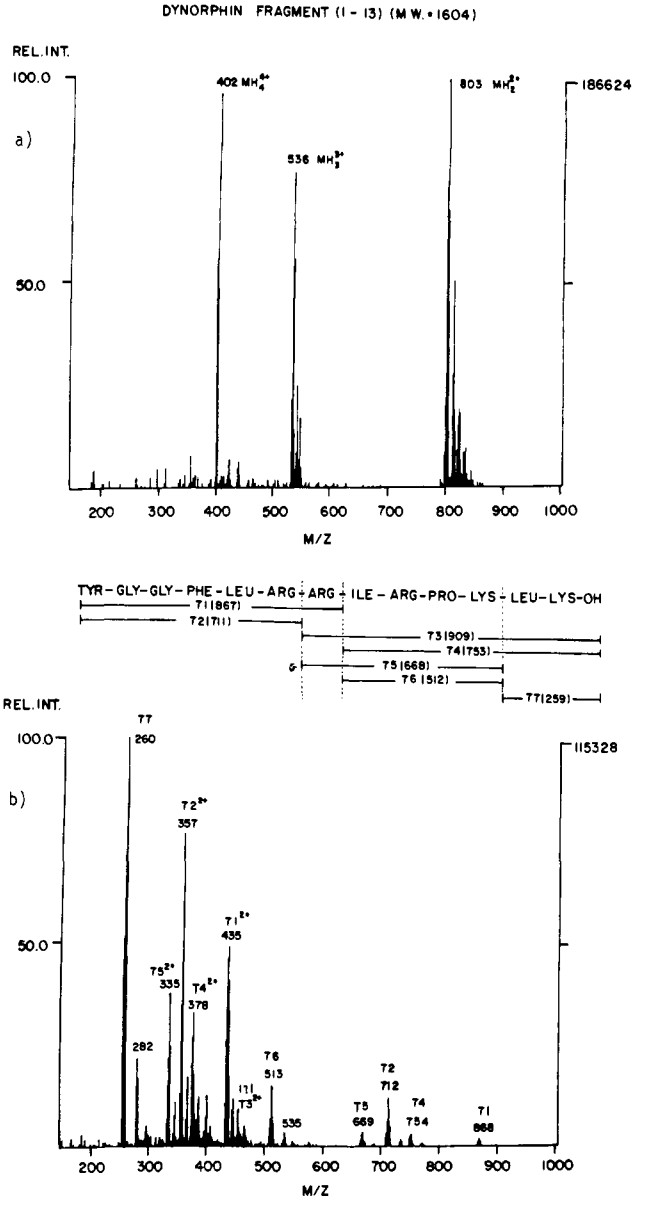

Figure 3. (a) A positive ion spectrum of a 2-nmol sample of dynorphin fragment 1–13 without enzymatic digestion. (b) The spectrum obtained after hydrolysis of the same peptide in a column containing 4.5×10^{-8} mol of immobilized trypsin, with the flow rate of 1.5 mL/min at room temperature. The sodium adducts appear 22 or 11 amu higher than the corresponding peptide peaks for singly or doubly charged ions, respectively.

arated tryptic fragments are carried directly into the thermospray ion source and detected. A typical example is shown for a tridecapeptide (MW 1604), fragment 1–13 of dynorphin. Figure 3a shows the spectrum obtained by injecting 2 nmol of sample without tryptic digestion, at pH 7.0. Doubly, triply, and quadruply charged molecular ions are shown at m/z 803, 536, and 402, respectively, along with their corresponding sodium and potassium adducts which show 11 and 19 mass units higher for doubly charged ion, and 7 and 13 mass units higher for triply charged ion. Although the appearance of multiply charged molecules for peptides does not exactly follow the solution chemistry, as reported for FD mass spectrometry,[32] presumably because of the gas-phase charge-transfer reaction in thermospray ionization process, the presence of five basic residues in the chain (three Arg and two Lys) allows generation of molecular ions with multiple positive charges at pH 7. Since the molecular weight of this compound is higher than the mass scan limit of our quadrupole analyzer (1200 amu), singly charged molecular ion species could not be detected.

After digestion by passage through a column containing 4.5×10^{-8} mol of immobilized trypsin (1.5 mL/min, 25 °C), the dynorphin sample generated the tryptic fragments which appear in Figure 3b. Trypsin hydrolyzes the C-terminus side of Arg or Lys in peptides, but does not cleave Arg-Pro or Lys-Pro. Under these conditions we could not observe the MH^+ of Arg at m/z 175. Except for T_3 with MW 909 (only tentatively identified as its MH_2^{2+} at m/z 456), all the expected peptide fragments were shown as doubly and/or singly charged ions. The results also indicate that hydrolysis of either of the two Arg cleavage sites is much faster than hydrolysis of the Lys-Leu bond. This is based on the fact that no mass ion corresponding to dynorphin fragment 1–11 could be observed. Apparently Lys-Leu hydrolysis occurs only subsequent to cleavage at one of the Arg sites in virtually all cases. An Arg peak, which corresponds to secondary hydrolysis product of fragments T_1, T_3, or T_5, could be observed only at higher temperature (35 °C) with a lower flow rate (1 mL/min).

Figure 4 shows the separation of tryptic fragments shown in Figure 3b. The Ultrasil-NH_2 column, which posesses a polar bonded phase, was eluted with 0.5 M ammonium acetate buffer (pH 7.0) at a flow rate of 1.5 mL/min. The chromatogram was obtained by full mass scanning from 200 to 1000 amu. Even though the separation was not perfect, selective ion recording for the mass ranges of interest permits the deconvolution of unresolved

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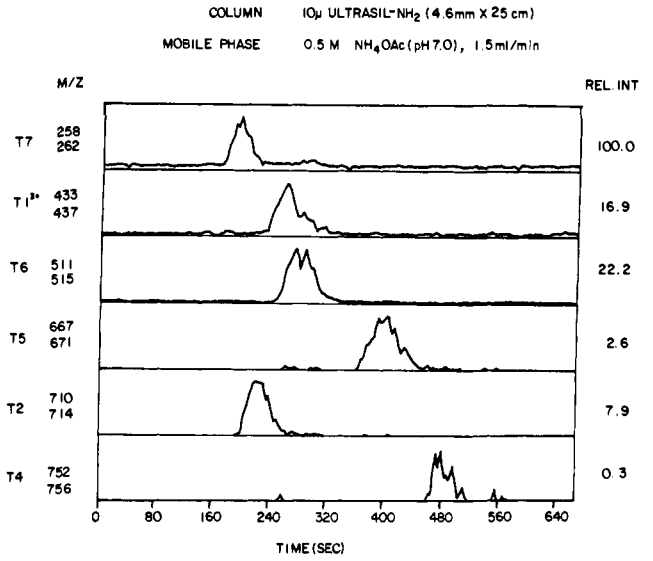

Figure 4. HPLC separation of the tryptic peptides shown in Figure 3b with an Ultrasil-NH_2 column, using 0.5 M NH_4OAc, pH 7.0, as a mobile phase. The mass ranges for each peptide were monitored by the selective ion recording mode from full mass scanning from 200 to 1000 amu.

chromatographic peaks of individual fragments. The mass ranges to be monitored can be selected from the mass spectrum of the tryptic peptide mixture for unknown samples. Separation allows more reliable detection of peptide fragments, often enabeling the detection of hidden peptide peaks from the background. Besides the rapidity and convenience usually obtained by HPLC analysis of tryptic peptides,[33] this technique also allows assignment of each component of the enzymatic digest, which adds an extremely important dimension to peptide mapping.

(3) On-Line Tryptic Digestion/HPLC Separation/Hydrolysis by CPY/Thermospray MS. In this application, immobilized trypsin, HPLC and immobilized CPY columns are connected in series and coupled to the thermospray mass spectrometer. The purpose of this approach is to develop an on-line sequencing technique with easy sample-handling procedures for large peptides, which will rapidly supply sequence information not only for terminal amino acid residues but also for multiple internal sites within the peptide chain. For purposes of clearity the application of this integrated system is illustrated in a stepwise manner on a sample peptide. This same stepwise approach can be applied in practice to simplify the analysis of experimental results.

The spectrum of tryptic products shown in Figure 5a was obtained by the passage of 2 nmol of a α-MSH (MW 1665) in pH 6.0, 0.5 M ammonium acetate buffer, through a column containing 4.5×10^{-18} mol of immobilized trypsin at 1.5 mL/min, room temperature. Since α-MSH has only one tryptic site, at -Arg-Trp-, only two tryptic fragments are expected. Indeed, all of the expected peaks showed up, at m/z 1099 and 550 for T_1 and at m/z 586 and 294 for T_2, as singly and doubly charged molecules, respectively. Bond cleavage was so fast that direct injection of the sample into the trypsin column under these conditions left no intact molecules, which otherwise would appear at m/z 833.5 and 556 as doubly and a triply charged molecular ions, respectively. The peak at m/z 719 is believed to arise from an impurity in the sample.

Figure 5b shows the chromatograms obtained by chromatography of the tryptic digest on the Ultrasil-NH_2 column. Both fragments T_1 and T_2 were eluted within 5 min using 0.5 M ammonium acetate buffer (pH 6.0) as an eluent with a flow rate of 1.2 mL/min. Although a better resolved chromatogram could be obtained using an organic modifier, an entirely aqueous buffer was required for maximum CPY activity in subsequent experiments, and is employed here as more representative of real experimental conditions. The coelution of the peaks at m/z 1099 and 550 confirms their assignment as differently charged forms of the same peptide and indicates how chromatographic separation can assist in the assignment of the multiply charged forms of peptides in more complex mixtures.

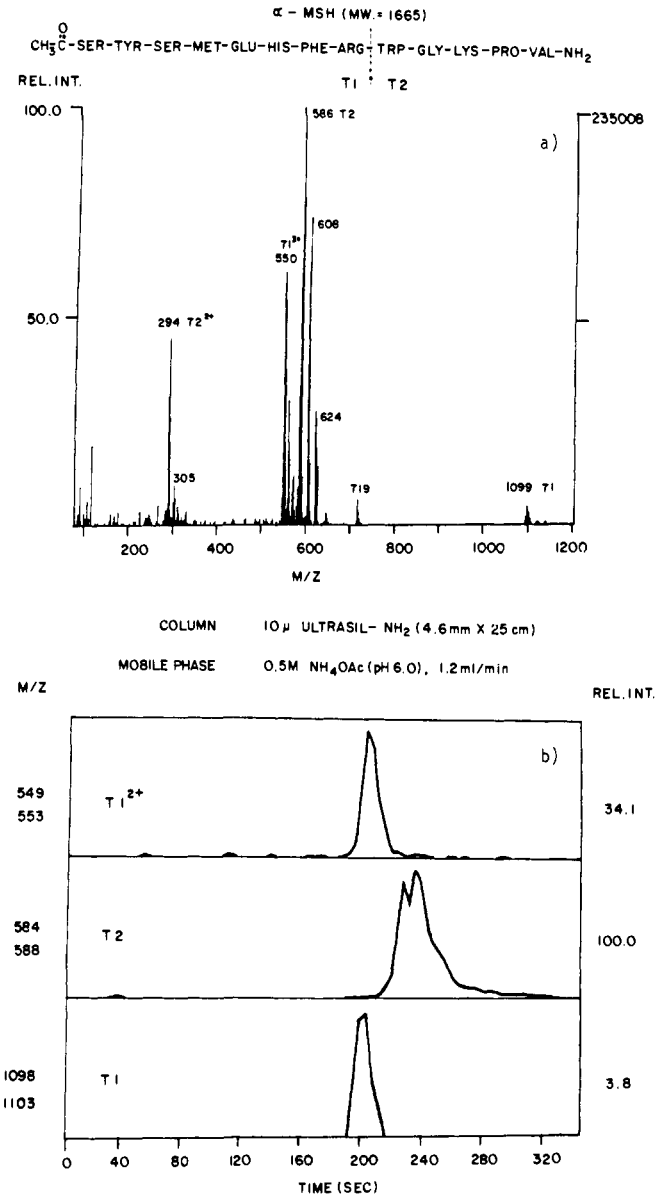

Figure 5. (a) A positive ion mass spectrum of a 2-nmol sample of α-MSH obtained after hydrolysis in a column containing 4.5×10^{-8} mol of immobilized trypsin at room temperature with a continuous flow of 0.5 M NH_4OAc, pH 6.0, and a flow rate of 1.2 mL/min. Two tryptic fragments T_1 and T_2 are shown as singly and doubly charged ions with their sodium and potassium ion adducts. (b) The results of separation of a digest done under the conditions described for Figure 5a. T_1 was monitored both as a singly and a doubly charged molecule.

In the fully integrated system the resolved tryptic peptides were subjected to on-line enzymatic hydrolysis by immobilized CPY at pH 6.0, and the mixtures of released amino acids and residual peptides were carried into the thermospray ion source by the continuous buffer flow. This is illustrated in the two spectra of Figure 6a and 6b, which were generated sequentially in a single experiment from tryptic peptides T_1 and T_2, respectively. As shown in Figure 6a cleavage of arginine was virtually complete, so the molecular ions previously observed at m/z 1099, 1121, and 1137 for the H^+, Na^+, and K^+ forms are no longer visible. Instead, each of the residual peptides resulting from the successive hydrolysis of Arg, Phe, His, Glu, Met, and Ser was observed, at m/z 943, 796, 659, 530, 399, and 311, respectively. The peaks from the cleaved amino acids also appear in the spectrum and have been identified. Detection of these amino acids gives additional con-

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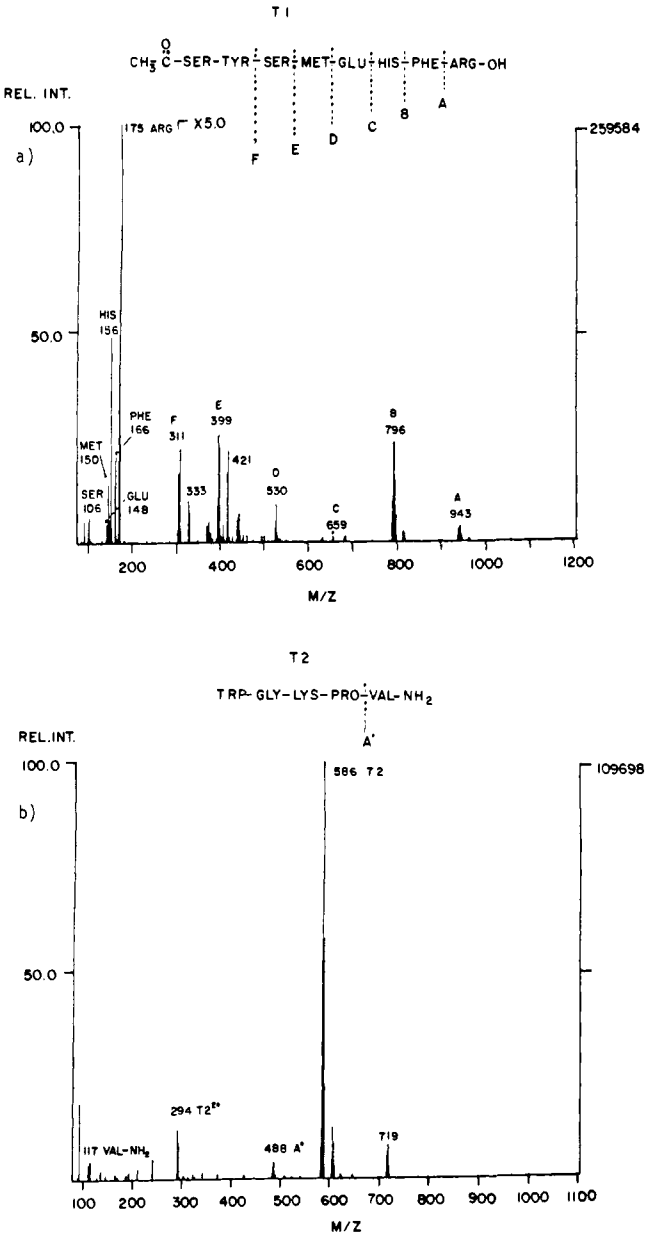

Figure 6. Positive ion spectra produced by hydrolysis of chromatographically separated T_1 and T_2 in a CPY column at room temperature. The buffer carrier, 0.5 M NH_4OAC, pH 6.0, flowed continuously at 1.2 mL/min. A, B, C, D, E, and F in (a) represent the residual peptides generated by hydrolysis of T_1 at A, B, C, D, E, and F, respectively. A′ in (b) also represents a residual peptide obtained by cleavage at A′ in T_2.

firmation to the assignments of the peaks from the residual peptides. For example, the peak at m/z 399 could represent the doubly charged form of peptide B (m/z 796) since the solution was not high enough to distinguish decimal mass unit differences, but, by a process of elimination based on the amino acids detected, this peak must represent the intermediate between peaks D and F and must arise from the release of methionine from D. (Note that the mass differences between sequence peptides must be 18 mass units less than the masses of the amino acids which are released.)

The residual peptide CH_3CO-Ser-Tyr-Ser-Met-Glu from T_1 (peptide C in Figure 6a) was rather difficult to observe, presumably because of slow cleavage of His and rather fast cleavage of Glu from their respective peptides at the given conditions. This relative cleavage rate can be controlled by changing the pH of the buffer solution, since each C-terminal amino acid has a different optimum pH for maximum enzyme activity.[34]

CPY also hydrolyzes amides very slowly at pH 6.0 as shown in Figure 6b. Only a small amount of residual peptide remaining after hydrolysis of Val-NH_2 was produced and appeared at m/z 488, while most of the T_2 molecule remains intact. The protonated molecule for Val-NH_2 was also detected. After the LC separation, a considerable decrease of sodium attachment was observed due to the removal of the alkali metals from the sample.

On-line operation using three columns in series, as described above, enables efficient samples utilization with great speed. By injecting 2 nmol of α-MSH just once, the sequence information for seven amino acids was generated in 5 min. Although a more refined separation technique is needed for complicated tryptic peptide mixtures, this technique can be extended to larger molecules which yield many tryptic fragments. Indeed, the main concern for those complicated cases is to find the best LC separating conditions without losing enzymatic activity.

Conclusion

The results described in earlier papers demonstrated the power of thermospray mass spectrometry for practical applications, not only for producing spectra of nonvolatile, thermally labile molecules, but also for detecting LC effluents with the conventional LC flow rate.[11-14] Subsequently the on-line operation of a thermospray mass spectrometer with enzymatic hydrolysis was achieved by immobilizing the enzymes on glass beads.

In this paper the combined, on-line application of enzyme and HPLC columns has been shown to be a practical method for the generation of useful peptide sequence information with great speed and reliability. Sensitivity is usually higher than (or at least comparable to) that of Edman method in most cases. In addition to the use of trypsin and CPY reported here, other endo- and exopeptidases such as chymotrypsin or aminopeptidases can be employed. Thus, this approach is generally applicable, in principle, to any kind of peptides or even proteins, in combination with proper separation techniques. The partial sequence information provided by one combination of enzymes can be added to that from a second and third pairing, and so on, such that in a relatively small number of experiments a large amount of sequence information can be generated, even from terminally blocked peptides. This information, even when incomplete, can be used in conjunction with gene-sequencing data for the identification of peptides and proteins, and in the search for peptides or proteins whose sequence is known but which have not yet been identified as gene products.

Finally, it should be noted that the results described in this paper have been obtained by the combination of two widely used laboratory instruments, the HPLC and the mass spectrometer, interfaced by readily prepared enzyme columns and the versatile thermospray ion source. Thus the capacity for rapidly obtaining peptide and protein sequence information without the purchase of a dedicated sequencing instrument is now available to many laboratories.

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Registry No. CPy, 9046-67-7; α-MSH, 37213-49-3; Phe-Leu-Glu-Glu-Ile, 62733-72-6; Trp-Gly-Lys-Pro-Val-NH_2, 65125-59-9; CH_3-CO-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg, 92543-12-9; trypsin, 9002-07-7; dynorphin (1-13), 72957-38-1.

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