Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 6367–6370, October 1985
Chemistry

Peptide mixture sequencing by tandem Fourier-transform mass spectrometry

(collisionally activated dissociation/gramicidin D/gramicidin S/laser-desorption ionization)

ROBERT B. CODY, JR.*, I. JONATHAN AMSTER†, AND FRED W. MCLAFFERTY†‡

*Nicolet Analytical Instruments, 5225 Verona Road, Madison, WI 53711; and †Chemistry Department, Cornell University, Ithaca, NY 14853-1301

Contributed by Fred W. McLafferty, May 29, 1985

ABSTRACT **Picomole samples of the linear peptide gramicidin D and cyclic peptide gramicidin S are shown to be impure by the laser-desorption formation of multiple groups of molecular adduct peaks by using Fourier-transform mass spectrometry. Selective excitation of the molecular peaks of the major sample component followed by collisionally activated dissociation provides complete sequence information for the cyclic decapeptide and for 12 of the 15 amino acids of the linear peptide. This instrumentation shows striking advantages in sensitivity, resolution, and mass accuracy in comparison to tandem mass spectrometers used previously.**

Mass spectrometry (MS) provides information complementary to conventional techniques for the molecular characterization of peptides and other important macromolecules (1–3). For oligopeptides, MS can utilize picomole samples, determine exact molecular weights, identify unusual amino acids or terminal groups (1–4), and detect frameshift errors in Maxam–Gilbert sequencing of the gene encoding an enzyme (5). MS characterization of larger peptides is now possible by using powerful methods for the ionization of nonvolatile molecules, such as fast atom bombardment (FAB) (2, 6–10), laser desorption (LD) (11–14), and particle-induced desorption (15, 16). The latter has recently been used to measure molecular ions of 23,406 ± 140 daltons from porcine trypsin (16). However, sequence information for larger peptides is often limited by the small degree of fragmentation observed (6–8, 11–13, 15, 16) and by misleading fragments arising from impurities; sample purity is also a critical limitation in conventional methods of peptide sequencing. Tandem mass spectrometry (MS/MS) offers a possible solution to both problems (17, 25, 26, 30). In MS/MS the molecular ion species of a mixture component can be separated by MS-I, fragmented by collisionally activated dissociation (CAD) (17) or laser photodissociation (18, 19) to produce fragments measured in MS-II indicating the peptide sequence (7, 8, 17–21, 25, 26, 30).

However, the few examples in which MS/MS has been applied to larger peptides (ionized by FAB) gave only limited sequence information of poor resolution (peak widths, >3 daltons), sensitivity (nmol samples), and mass accuracy (7, 8, 17, 25, 26). This arises because primary ion dissociations are accompanied by translational energy release, causing both broadening and shifting of peaks in magnetic sector instruments (17). Fourier-transform mass spectrometry (FTMS) (22–24) offers a promising alternative for such studies. The measured cyclotron frequency only depends on the magnetic field and ion's mass, not on its translational energy. Further, the masses of nearly all (e.g., m/z 100–16,000, broad-band recording) ions can be measured simultaneously, so that pulsed ionization is feasible; scanning instruments require continuous ionization, at any instant wasting all ions formed except those of the exact mass measured. For modern FTMS instrumentation we have recently demonstrated high (>16,000) mass range and unusually high (150,000 at m/z 1180, narrow-band recording) resolution. Further, the ion measurement cell can be reused to effect MS/MS; after forming a mixture of molecular ions, those desired can be selectively excited for CAD (or others ejected) by using the appropriate cyclotron frequencies (23, 27, 28). We report here on the sequence information derivable from the major components of the linear and cyclic peptides gramicidins D and S by the use of FTMS/MS.

EXPERIMENTAL

The Nicolet FTMS-1000 instrument employed a 3-tesla magnet and a Tachisto model 215G pulsed infrared laser for desorption ionization of the sample (mixed with an excess of KBr) placed at an entrance to the single ion cell; further details are given elsewhere (14, 24). Although no attempt was made to determine the minimum sample requirements, the hole left from the laser shot corresponds to a few picomoles of sample desorbed. The 10^6–10^7 ions trapped in the cell from a single laser pulse are used to measure the primary ion spectrum. Alternatively, specific primary ions can be selectively (resolution, ≈1000) accelerated to a maximum orbit by using their cyclotron resonance frequency and dissociated (CAD) by admitting argon through a pulsed valve. The gramicidin D and S samples were used as obtained from Sigma.

RESULTS AND DISCUSSION

Linear Peptides. LD of the antibiotic gramicidin D, consuming a few picomoles of sample, gave the mass spectrum of Fig. 1. Alkali ion attachment produces the $[M + K]^+$ molecular ions of four mixture components as the major ions. Each gives a cluster of isotopic peaks; the major component has the composition $C_{99}H_{140}N_{20}O_{17}K$, so that the 1.1% relative abundance of ^{13}C causes m/z 1921 to be of higher abundance than m/z 1920. This "soft" ionization has caused little molecular ion fragmentation; m/z 1902–1905 is probably due to water loss from m/z 1920–1923, and peaks of lower masses are <5% relative abundance. From the known composition (29) of this naturally occurring mixture the m/z 1920 cluster can be assigned the composition Val-1 gramicidin A (1920.034), m/z 1934 as its Ile-1 isomer, and m/z 1881 and 1897 as its Phe-11 and Tyr-11 (replacing Trp) isomers.

Selective CAD of $[M + K]^+$ ions of the major component, mainly m/z 1920 and 1921, produces the MS/MS spectrum of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MS/MS, tandem mass spectrometry; FTMS, Fourier-transform mass spectrometry; FAB, fast atom bombardment; CAD, collisionally activated dissociation; LD, laser desorption.

‡To whom reprint requests should be addressed.

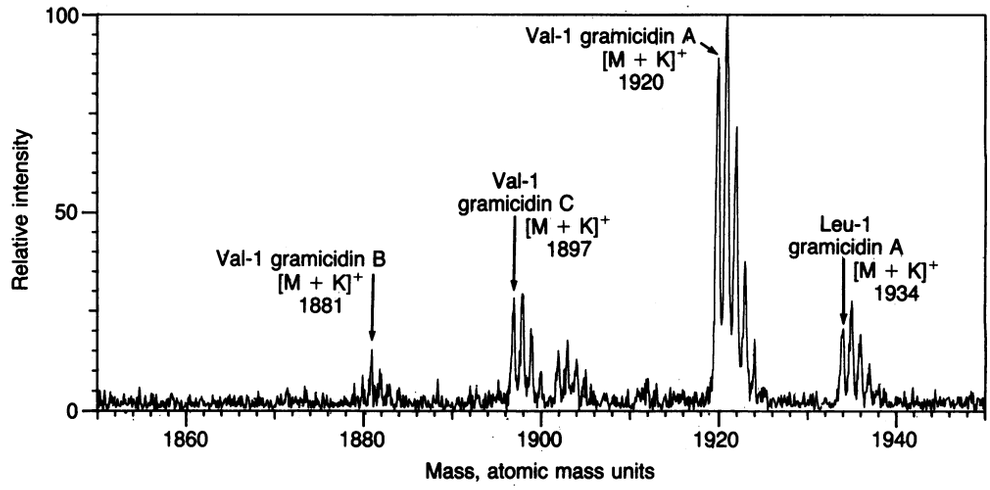

FIG. 1. FT mass spectrum from LD ionization of gramicidin D mixed with KBr, indicating four mixture components.

Fig. 2. Thus, most high-mass fragment peaks are isotopic doublets with the computer-assigned mass referring to the peak of higher abundance. The mass differences between most of these peaks can then be assigned to masses of the common amino acid units. Thus, the masses 1737, 1666, 1552–1553, 1481–1482, 1381–1382 (one dalton low), 1283–1284, 1184–1185, 998–999, 884–885, 698, 585, 399 correspond to losses of Ala (71), Leu (or Ile, 113), Ala, Val (99), Val, Val, Trp (186), Leu, Trp, Leu, Trp, providing the sequence of 11 of the 15 amino acids. This series corresponds to cleavages from the N-terminus with rearrangement of a hydrogen (the exact masses 997.507 and 884.423 account for

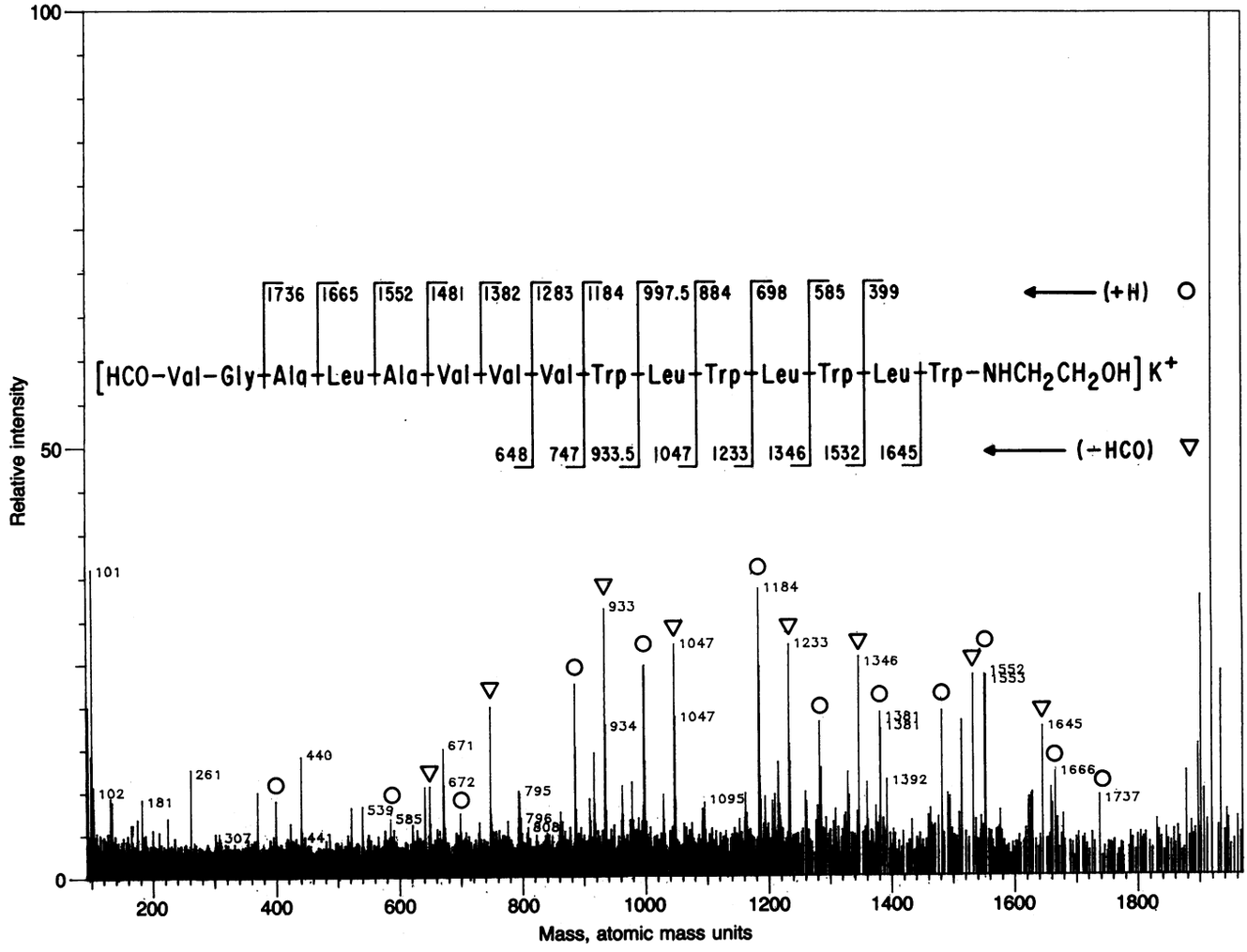

FIG. 2. FT mass spectrum resulting from the selective CAD of the m/z 1920–1921 ions of Fig. 1 representing the major mixture component. ○ and ▽, the sequence peaks containing the C- and N-termini, respectively.

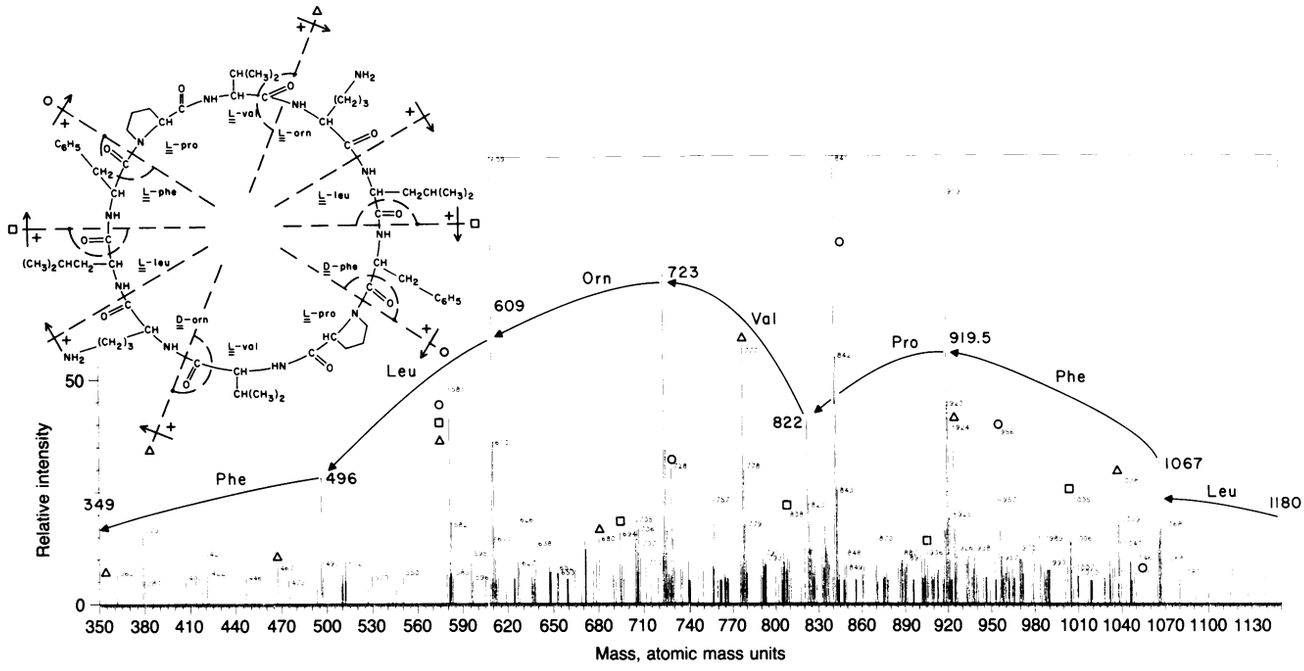

FIG. 3. FT mass spectrum resulting from the selective CAD of the m/z 1180–1182 ions from the LD ionization of gramicidin S. Formation of four of the five possible sets of sequence peaks is shown in the structure and explained in the text.

the leucine mass difference of 113). Cleavages from the C-terminus after loss of the N-terminal HCO give confirmatory sequence evidence and indicate a 12th residue, as shown in Fig. 2. These two sequences account for most of the major peaks except at low masses; when the dual-cell modification is available (24) these remaining peaks can be investigated further by using exact mass data to provide their elemental compositions.

Cyclic Peptides. LD ionization of the antibiotic gramicidin S gave mainly the $[M + K]^+$ peak m/z 1180 and its isotopic peaks (with the dual cell we have measured these recently with 150,000 resolution). Minor peaks include 10% $[M + Na]^+$ and 10% m/z 1152/1153 and 5% 1194/1195, which could be impurities in which alanine and leucine, respectively, replace valine. Peaks of lower mass are <5% abundance. CAD of m/z 1180–1182 produces the MS/MS spectrum of Fig. 3. Sequencing of cyclic peptides is complicated by the fact that a multiplicity of initial ring cleavages is possible, each of which can produce a series of sequence peaks. The first major fragment peak at m/z 1067 involves the loss of 113 daltons, indicative that this ring cleavage produces an N-terminal leucine. The major m/z 920 peak represents the subsequent loss of Phe (mass 147), followed by the loss of Pro (97) to give the significant m/z 822 peak (actually, 919.517 → 822.464). Similarly, the peaks at 723, 609, 496, 349, and 252 (not shown) correspond to the losses of Val (99), Orn (114), Leu (or Ile), Phe, and Pro. In the initial Orn-Leu cleavage, stabilization of the positive charge on the ornithine apparently is favored by the presence of its amino group, but ring cleavage at other amide bonds apparently is followed by CO loss. Thus, the significant m/z 1038 peak appears to arise from Val-Orn cleavage with CO loss from the valine to stabilize the positive charge on its amide nitrogen (an "α-cleavage"); the m/z 1180, 1038, 924.5, 777, 680, 581, 467, 354 peaks are indicative of the sequence Orn-Leu-Phe-Pro-Val-Orn-Leu. Two other such initial ring cleavages accompanied by CO loss are shown in Fig. 3 and account for most of the major remaining ions in the MS/MS spectrum. An obvious advantage of the unit mass accuracy and resolution in the FTMS/MS spectrum is that the mass difference of 114 due to the unusual amino acid ornithine is not confused with the 113 mass difference of leucine.

Future. The mass range of >16,000 daltons and the high-resolution capabilities of the dual cell (24) can be exploited in continuing work. Exact mass measurement of the low-mass peaks could serve to identify terminal groups and individual amino acids; MS/MS/MS could differentiate isomers such as Leu/Ile (21). Microprobe techniques can be investigated to minimize sample requirements; the current estimated picomole sample vaporized corresponds to an ionization and trapping efficiency of only $1/10^5$.

We are grateful to D. P. Littlejohn, S. Ghaderi, and J. A. Loo for helpful discussions and to the National Institutes of Health (Grant GM16609) for generous financial support.

1. Arpino, P. J. & McLafferty, F. W. (1976) in *Determination of Organic Structures by Physical Methods*, eds. Nachod, F. C., Zuckerman, J. J. & Randall, E. W. (Academic, New York), Vol. 6, pp. 1–89.
2. Morris, H. R., ed. (1981) *Soft Ionization Biological Mass Spectrometry* (Heyden, London).
3. Desiderio, D. M. (1984) *Analysis of Neuropeptides by Liquid Chromatography and Mass Spectrometry* (Elsevier, Amsterdam).
4. Carr, S. A., Biemann, K., Shoji, S., Parmelee, D. C. & Titani, K. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6128–6131.
5. Putney, S. D., Royal, N. J., DeVegvar, H. N., Herlihy, W. C., Biemann, K. & Schimmel, P. (1981) *Science* **213,** 1497–1501.
6. Barber, M., Bordoli, R. S., Elliott, G. J., Sedgwick, R. D. & Tyler, A. N. (1982) *Anal. Chem.* **54,** 645A–657A.
7. Amster, I. J., Baldwin, M. A., Cheng, M. T., Proctor, C. J. & McLafferty, F. W. (1983) *J. Am. Chem. Soc.* **105,** 1654–1655.
8. Barber, M., Bordoli, R. S., Elliott, G. J., Tyler, A. N., Bill, J. C. & Green, B. N. (1984) *Biomed. Mass Spectrom.* **11,** 182–186.
9. Morris, H. R., Pamico, M., Etienne, T., Tippins, J., Girgis, S. I. & MacIntyre, I. (1984) *Nature (London)* **308,** 746–748.
10. Stroh, J. G., Cook, J. C., Milberg, R. M., Brayton, L., Kihara, T., Huang, Z. & Rinehart, K. L., Jr. (1985) *Anal. Chem.* **57,** 985–991.
11. Posthumus, M. A., Kistemaker, P. G., Meuzelaar, H. L. C. & Ten Noever de Brauw, M. C. (1978) *Anal. Chem.* **50,** 985–991.

12. McCreary, C. A., Ledford, E. B., Jr., & Gross, M. L. (1982) *Anal. Chem.* **54,** 1435–1437.
13. Cotter, R. J. (1984) *Anal. Chem.* **56,** 485A–504A.
14. Wilkins, C. L., Weil, D. A., Yang, C. L. C. & Ijames, C. F. (1985) *Anal. Chem.* **57,** 520–524.
15. Macfarlane, R. D. (1982) *Acc. Chem. Res.* **15,** 268–275.
16. Sundqvist, B., Roepstorff, P., Fohlman, J., Hedin, A., Hakansson, P., Kamensky, I., Lindberg, M., Salehpour, M. & Sawe, G. (1984) *Science* **226,** 696–698.
17. McLafferty, F. W., ed. (1983) *Tandem Mass Spectrometry* (Wiley, New York).
18. Dunbar, R. C. (1979) in *Gas Phase Ion Chemistry*, ed. Bowers, M. T. (Academic, New York), pp. 182–220.
19. McIver, R. T., Jr., Bowers, W. D., Delbert, S.-S. & Hunter, R. L. (1984) *J. Am. Chem. Soc.* **106,** 7288–7289.
20. Wipf, H.-K., Irving, P., McCamish, M., Venkataraghavan, R. & McLafferty, F. W. (1973) *J. Am. Chem. Soc.* **95,** 3369–3375.
21. Levsen, K., Wipf, H.-K. & McLafferty, F. W. (1974) *Org. Mass Spectrom.* **8,** 117–128.
22. Comisarow, M. B. & Marshall, A. G. (1974) *Chem. Phys. Lett.* **25,** 282–283.
23. Gross, M. L. & Rempel, D. L. (1984) *Science* **226,** 261–268.
24. Settine, R. L., Kinsinger, J. A. & Ghaderi, S. (1985) *Eur. Spectrosc. News* **58,** 16–18.
25. Derrick, P. J. & Neumann, G. M. (1984) *Org. Mass Spectrom.* **19,** 165–170.
26. Tomer, K. B., Crow, F. W., Gross, M. L. & Kopple, K. D. (1984) *Anal. Chem.* **56,** 880–886.
27. Cody, R. B., Jr., & Freiser, B. S. (1982) *Anal. Chem.* **54,** 1431–1433.
28. White, R. L. & Wilkins, C. L. (1982) *Anal. Chem.* **54,** 2211–2215.
29. Hunter, F. E., Jr., & Schwartz, L. S. (1967) in *Antibiotics*, eds. Gottlieb, S. & Shaw, P. (Springer, New York), Vol. 1, pp. 642–648.
30. Lippstreu-Fisher, D. L. & Gross, M. L. (1985) *Anal. Chem.* **57,** 1174–1180.