Comparison of the Fragmentation Produced by Fast Atom Bombardment and Photodissociation of Peptides

Carlito B. Lebrilla, Diana T.-S. Wang, Tadashi J. Mizoguchi, and Robert T. McIver, Jr.*

Contribution from the Department of Chemistry, University of California, Irvine, California 92717. *Received April 17, 1989*

Abstract: Laser photodissociation has many features that make it an attractive alternative to collision-induced dissociation (CID) for sequencing peptides by mass spectrometry. In this paper we report on the photodissociation at 193 nm for two tetrapeptides (Val-Ala-Ala-Phe and Met-Arg-Phe-Ala) and a cyclic peptide, gramicidin S. The experiments are performed by using an external ion source Fourier transform mass spectrometer (FTMS) and an excimer laser. Ions are generated by particle beam bombardment and are transferred to an FTMS analyzer cell where they are trapped. While trapped in the analyzer cell, the ions are irradiated with pulsed UV light from an excimer laser. Photodissociation is very efficient at 193 nm. Only a single laser pulse is required to fragment about 60% of the parent ions. Fragmentation results mainly in cleavage of the peptide bonds and produces easily interpretable mass spectra. Abundant fragment ions are produced by residues that contain high proton affinity amino acids such as proline. This indicates that the mechanism of peptide photodissociation is controlled primarily by thermodynamic factors rather than photochemical factors such as the molar absorption coefficients of the various amino acids in the chain.

Dramatic progress has been made recently in the development of new methods for the ionization and mass analysis of peptides and small proteins by mass spectrometry. Karas and Hillenkamp have used a reflector-type time-of-flight mass spectrometer and a Nd:YAG laser to desorb ions for a number of proteins, up to bovine albumin at 67 000 daltons.[1] The samples were dispersed in a matrix of nicotinic acid which served to absorb the 266-nm laser light. Macfarlane and co-workers have developed a method called plasma desorption mass spectrometry (PDMS) which uses fission fragments from the decay of ^{252}Cf to desorb and ionize high molecular weight organic molecules deposited on a surface.[2,3] One of the most impressive accomplishments to date was a report which used PDMS to determine a molecular weight of 23 463 for porcine trypsin.[4,5] Another approach is the electrospray ionization method used by Fenn and co-workers to generate multiply charged small proteins up to 40 000 daltons.[6,7] The most widely used method for peptides is fast atom bombardment (FAB), or liquid secondary ion mass spectrometry (LSIMS), in which the sample is dissolved in a liquid matrix such as glycerol and is bombarded with high-energy (10–30 keV) particles.[8,9] FAB has been used successfully to analyze small proteins in the range 10 000 to 24 000 daltons in a conventional double-focusing mass spectrometer.[10]

What is remarkable about FAB and the other ionization methods mentioned above is that the mass spectra contain strong peaks for protonated, or multiply protonated, intact molecular ions.[11-13] These peaks are useful for determining the molecular weight of a peptide, but often there are not enough fragment peaks to determine the amino acid sequence. One approach to solving this problem is to use a tandem mass spectrometer to fragment the parent ions by passing them through a gas collision cell. This technique, called collision-induced dissociation (CID), works well for low-mass peptides, but recent evidence suggests that high mass ions are not efficiently fragmented by CID because of the increasing amount of energy that a large molecule can accommodate before dissociating (the number of vibrational modes increases) and the decreasing amount of energy that can be transferred during a collision (center of mass effect).[14,15]

Laser photodissociation has many features that make it an attractive alternative to CID for obtaining more structural information. Earlier we reported that small peptides are efficiently dissociated by ultraviolet (UV) light (193-nm wavelength) from an excimer laser.[16,17] Our experiments were performed by using a Fourier transform mass spectrometer (FTMS) to store the ions and detect the daughter ions produced by photodissociation. It would be difficult to do these experiments with a quadrupole or sector mass spectrometer because UV lasers produce very short pulses (20 ns) and have low repetition rates (10–200 pulses/s). Such a low-duty cycle (0.0001%) is incompatible with the continuous beam, scanning mode of operation that is normally used with quadrupole and sector mass spectrometers. In contrast, FTMS is well suited for pulsed laser experiments because ions can be stored in the analyzer cell for several seconds and irradiated with many laser pulses. At the end of the laser irradiation period, a complete mass spectrum of the laser-produced daughter ions can be obtained.

Another capability that we demonstrated is that selected peptide daughter ions can be isolated in the FTMS analyzer cell and subsequently photodissociated by a second laser pulse to give granddaughter ions, as shown below.[17]

$$\text{parent} \xrightarrow{h\nu} \text{daughter} \xrightarrow{h\nu} \text{granddaughter} \qquad (1)$$

This concept is similar to the MS/MS/MS experiments done in tandem mass spectrometry, but multiple laser pulses are utilized to fragment the ions rather than multiple gas collision cells.

Although the combination of FTMS and laser photodissociation offers many potentially attractive features for structural analysis of high mass ions, progress has been slow because of the difficulty of interfacing FTMS with the types of ionization methods that produce high mass ions. For example, the glycerol matrix used

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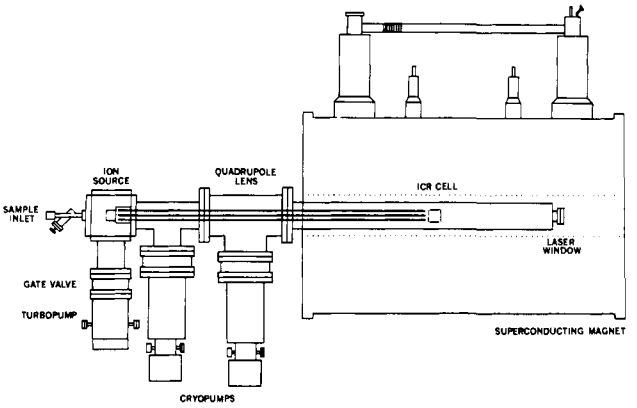

Figure 1. Schematic drawing of a Fourier transform mass spectrometer with an external ion source.

in FAB causes difficulties because its vapor pressure (about 10^{-5} Torr) is too high. The FTMS detection method works best at pressures below 10^{-7} Torr, but at 10^{-5} Torr the mass resolution and sensitivity of the method are severely degraded. These problems can only be overcome by separating the ion source from the FTMS analyzer cell. This was first accomplished by McIver and co-workers in 1983 by using an FTMS instrument with an external ion source.[18-20] With this instrument, ions are produced by a quadrupole mass spectrometer (Q1) and guided by a second quadrupole mass filter (Q2) to a FTMS analyzer cell inside the bore tube of a superconducting magnet. The first experiments were done with an electron ionization source.[18] Subsequent work in collaboration with Hunt and Shabanowitz demonstrated that high-mass resolution could be obtained for peptides ionized by FAB.[21,22]

In this paper we report the photodissociation mass spectra for two tetrapeptides (Val-Ala-Ala-Phe and Met-Arg-Phe-Ala) and a cyclic peptide, gramicidin S. By comparing these spectra with ones produced by FAB, the internal energy of the ions and the mechanism of fragmentation can be elucidated.

Experimental Section

The fast atom bombardment and laser photodissociation experiments reported in this paper were performed with a new external ion source Fourier transform mass spectrometer that is shown in Figure 1. The instrument has three regions: an external ion source where sample ions are produced, an ion transport region with a long quadrupole lens, and an ion detection region with an ion cyclotron resonance (ICR) analyzer cell where the ions are trapped and FTMS detection is performed. The ion source region consists of a stainless steel cube, a gate valve, and a turbomolecular pump (Balzers TPU 170). Peptide samples dissolved in 5% acetic acid are placed on a glycerol-covered probe tip and bombarded by 10-keV cesium ions. The cesium ion gun (Antek, Palo Alto, CA) is pulsed on for 50 ms and the peak current is about 10 μA.[23,24] The angle of the cesium ion beam with respect to the probe surface is 45°.

Secondary ions are extracted from the source through a 1.6-mm diameter hole and accelerated into a long quadrupole lens assembly that extends 117 cm from the ion source to the ICR cell. A high-voltage radio-frequency signal (400 to 600 V, 1 MHz) is applied to the quadrupole rods to focus the ions into a tight beam and guide them through the fringing fields of the superconducting magnet.[25] The rf-only operating mode for the quadrupole also causes it to function like a "band-pass filter" with a variable low-mass and high-mass cutoff. Typically, operating conditions are set so that ions over a rather wide range, from m/z 100 to m/z 5000, are transmitted to the analyzer cell. An Air Products cryopump evacuates the ion transport region, and a second cryopump evacuates the analyzer region to a base pressure of 5×10^{-10} Torr. A pressure gradient of 40000:1 is maintained between the ion source and the analyzer cell.

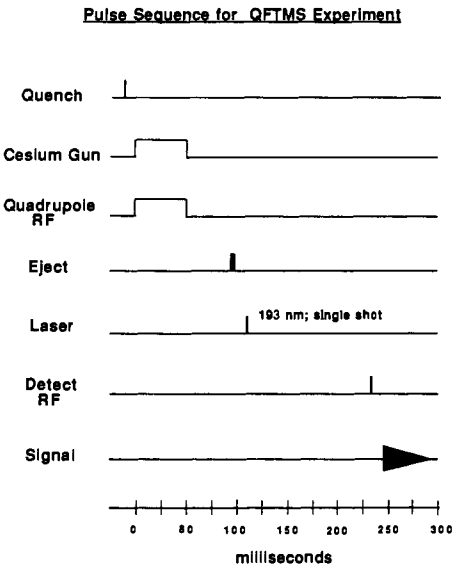

Figure 2. Pulse sequence for a FTMS photodissociation experiment. Ions are formed when the sample is bombarded by a pulsed cesium ion beam. The ions are injected into a FTMS analyzer cell, and sweep-out pulses are used to isolate a particular peak of interest. After the laser fires, the daughter ions formed by photodissociation are detected.

The ICR analyzer cell shown in Figure 1 measures 5 × 5 × 5 cm and is positioned in the homogeneous region of a 6-T superconducting magnet made by Oxford instruments. The room-temperature bore of the magnet has a diameter of 15 cm. A magnesium fluoride window on a Conflat flange is mounted on the end of the vacuum manifold so that ions trapped in the analyzer cell can be irradiated with a laser beam. Laser photodissociation experiments are performed by using an excimer laser (Lambda Physik Model 201) operated with ArF at 193 nm and 20 to 50 mJ per pulse. The beam was unfocused and apertured to a diameter of 3.0 cm. The resulting laser power density, about 100 kW/cm^2, is low enough so that multiphoton ionization processes are not observed.

The FTMS experiments are controlled by an OMEGA data system (IonSpec Corp., Irvine, CA). Unless otherwise noted, all mass spectra were obtained in the broad-band mode. This mode is used to detect ions over a wide mass range, which with our present data system extends from m/z 93 to m/z 30000. Another method, called narrow-band acquisition, is used to detect ions below m/z 93 and to acquire high-mass resolution data over a narrow range. Narrow-band acquisition uses heterodyne signal processing circuitry to convert high-frequency cyclotron resonance signals to the audio-frequency range so that they can be digitized more easily.

A typical pulse sequence for operation of the external ion source FTMS instrument is shown in Figure 2. An experiment is initiated by a quench pulse that changes the voltage on one of the analyzer cell trapping plates from +1.5 V to −12 V. This clears out all ions from the analyzer cell. After a few milliseconds the cesium ion beam is pulsed on and at the same time the rf voltage for the quadrupole lens is turned on. Secondary ions are extracted continuously from the ion source and guided by the quadrupole lens to the analyzer cell where a small fraction of them get trapped. After 50 ms the cesium ion beam and quadrupole rf voltage are turned off. At this point a FTMS detect pulse can be triggered to acquire a mass spectrum showing the ions produced by FAB ionization. For photodissociation studies, however, the detect pulse is delayed longer and eject pulses are used first to isolate ions of a particular mass. The isolated ions are then irradiated by one or more laser pulses, and a FTMS mass spectrum of the daughter ions is acquired. This cycle is automatically repeated and the FTMS signals can added together to improve the signal-to-noise ratio.

Val-Ala-Ala-Phe, Met-Arg-Phe-Ala and gramicidin S were obtained from Sigma and were used without further purification. Solutions of the peptides (1 μg/μL) were prepared in 5% acetic acid. The samples were prepared for FAB ionization by putting 1 μL of glycerol on the end of a copper probe tip and then adding 1 μL of the peptide solution to the

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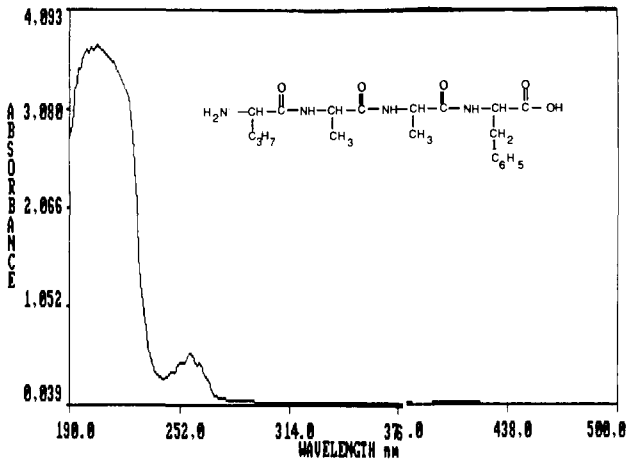

Figure 3. Absorption spectrum of Val-Ala-Ala-Phe in aqueous solution.

surface of the glycerol droplet. For the UV-visible spectrum of Val-Ala-Ala-Phe a solution containing 1.2 mg/mL in water was prepared.

Results and Discussion

Although photodissociation has proven to be a powerful method for studying the spectroscopy, structure, and thermochemistry of low-mass ions,[26-28] only a few studies have reported its use to fragment high molecular weight ions.[16,17,29-32] Welch and co-workers reported that peptide ions generated by FAB were not photodissociated by visible light from a CW argon ion laser, even when the full 18-W output of the laser was used.[33] The reason for this can be understood by examining Figure 3, which is the UV-visible absorption spectrum of Val-Ala-Ala-Phe in water. This spectrum shows that the peptide does not absorb appreciably in the visible and near-UV regions. The first absorption begins at about 260 nm due to the π to π^* transition in the phenyl group of Phe. At shorter wavelengths the molar absorption coefficient increases sharply due to n to σ^* transitions in the carbonyl group and higher π to π^* transitions in the phenyl groups. Thus under the low fluence conditions of a CW laser, where single photon absorption dominates and multiphoton processes are minimal, photodissociation of peptides is not efficient because the laser light in the visible range is not absorbed. In contrast, UV laser light between 190 and 260 nm is strongly absorbed and would be expected to be most effective for photodissociating peptide ions. This is the reason we chose to use an excimer laser (ArF, 193 nm wavelength) for our initial photodissociation experiments.[16,17]

In the following sections, results are presented for UV laser photodissociation of three peptides, and the fragment ions produced are compared with those made directly by the FAB ionization process.

Val-Ala-Ala-Phe. Three FTMS mass spectra obtained for Val-Ala-Ala-Phe are shown in Figure 4. Figure 4a shows the ions that are produced by FAB and injected into the analyzer cell. Not shown in this spectrum is a large peak for m/z 71 that was detected using the narrow-band acquisition mode. At the bottom of Figure 4 the structure of Val-Ala-Ala-Phe is given and the peak assignments for the FAB-produced ions are shown above the structure. There is a prominent peak for the protonated molecular ion at m/z 407, and the most abundant fragment ions result from

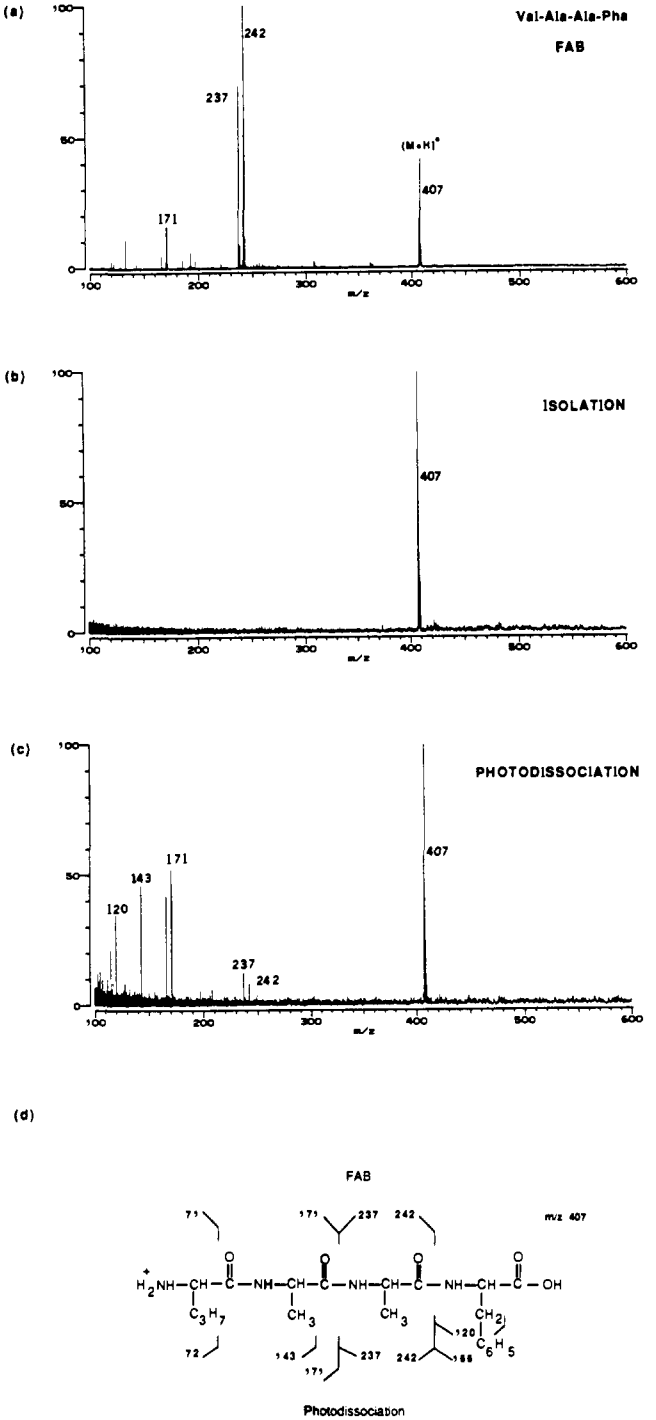

Figure 4. FTMS mass spectra for Val-Ala-Ala-Phe and the peak assignments for the fragment ions: (a) ions produced by 10-keV cesium ion bombardment of the sample dissolved in glycerol, (b) mass spectrum obtained after sweep-out pulses are used to isolate the protonated parent ion, (c) daughter ions produced by photodissociation of m/z 407 with a single laser pulse (wavelength 193 nm and 20 mJ).

cleavage of the peptide bonds. The fragment ions at m/z 71, 171, and 242 are the A_1, A_2, and A_3 sequence ions, respectively.[36] The large peak at m/z 237 is the Y_2 sequence ion containing the C-terminus.

For the photodissociation experiments, sweep-out pulses are used to eject all ions from the analyzer cell except the ones of interest. Figure 4b is an example of this and shows isolation of the protonated molecular ion peak at m/z 407. Conceptually this is the same as the daughter ion scan mode in tandem mass spectrometry,

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where the first mass analyzer is set to pass only a particular parent mass.[37]

After the ions are isolated, the laser is fired once and a mass spectrum of the daughter ions, Figure 4c, is obtained. More than one laser pulse could be used if necessary, but for this peptide one pulse is sufficient to dissociate about 60% of the parent ions. The peak assignments for the photodissociation fragment ions are shown at the bottom of the Val-Ala-Ala-Phe drawing. The major photodissociation fragment (greater than 50% relative abundance) is m/z 72 due to the N-terminal valine. This peak is not shown in Figure 4c because it was detected separately using the narrow-band detection mode. Comparison of Figure 4a and 4c shows that the photodissociation spectrum has essentially all the peaks that are in the FAB spectrum. The most noticeable difference in that the relative abundances of m/z 237 and 242 in the photodissociation spectrum is much smaller than in the FAB spectrum. On the other hand, there are several peaks in the photodissociation spectrum that are not present in the FAB spectrum. For example, a large peak at m/z 166 is the Y_1 sequence ion (the C-terminal Phe), and m/z 120 is the phenylalanylimmonium ion ($C_6H_5CH_2CH_2{=}NH_2^+$). Photodissociation also gives a large peak for the A_2 sequence ion at m/z 143. The site of greatest basicity in this peptide is certainly the N-terminal valine. This is confirmed in both the FAB and the photodissociation spectra by the large relative abundance of fragment ions which retain the N-terminus.

Met-Arg-Phe-Ala. The FAB mass spectrum for Met-Arg-Phe-Ala is shown in Figure 5a, and Figure 5b is the photodissociation spectrum for the protonated molecular ion. The structure of the peptide and the peak assignments are given at the bottom of the figure. As expected, the FAB spectrum shows a strong protonated molecular ion peak, m/z 524, and a number of sequence ions that result from cleavage at or near the peptide bonds. For example, the N-terminal Met is identified by the A_1 and B_1 fragments at m/z 104 and 133, respectively. Although some of the peak at m/z 133 might be due to Cs^+ from the primary ion beam rather than from Met, there is usually not much Cs^+ trapped in the analyzer cell (see Figure 4). This probably occurs because the 10-keV Cs^+ ions, which have a high translational energies as they scatter off the glycerol droplet, are not well transmitted by the quadrupole lens and are not low enough in energy to be trapped in the analyzer cell. Figure 5a also shows large peaks for the B_2 sequence ion, Met-Arg, at m/z 288 and 271. From the C-terminus, the X_1 ion at m/z 117, and Y_2 ion at m/z 237, and the Y_3 ion at m/z 393 are evident.

For comparison, Figure 5b shows the mass spectrum obtained following isolation and photodissociation of the protonated parent ion, m/z 524. The photodissociation spectrum shows peaks for the A_1 (m/z 104) and B_2 (m/z 288 and 271) N-terminal sequence ions. From the C-terminus, the X_1 ion (m/z 117) from the terminal Ala is larger than in the FAB spectrum, but the peak for the Y_2 ion (m/z 237) is very weak and there is no signal for the Y_3 ion (m/z 393). Photodissociation of Met-Arg-Phe-Ala produces several peaks due to fragmentation of the side chains. For example, m/z 112 is from Arg, and m/z 120 is the same phenylalanylimmonium ion from Phe that was observed in the photodissociation of Val-Ala-Ala-Phe. The absence of a peak at m/z 166, which was present in the photodissociation spectrum of Val-Ala-Ala-Phe, indicates that the phenylalanine is not on the C-terminal position.

It is apparent from these two mass spectra that the UV laser photodissociation of Met-Arg-Phe-Ala produces a larger relative abundance of low-mass ions than FAB. This is not particularly useful for structure elucidation and suggests that the internal energy of the protonated molecular ions made by FAB is lower than the 6.4 eV of internal energy that is added by adsorption of one photon of 193-nm light.

Gramicidin S. The two previous examples compare the FAB spectrum of a peptide with the photodissociation mass spectrum for its protonated molecular ion. It is also possible, however, to isolate one of the FAB-produced fragment ions and photodissociate it. This is illustrated in Figure 6 for m/z 571 that is formed by FAB ionization of gramicidin S.

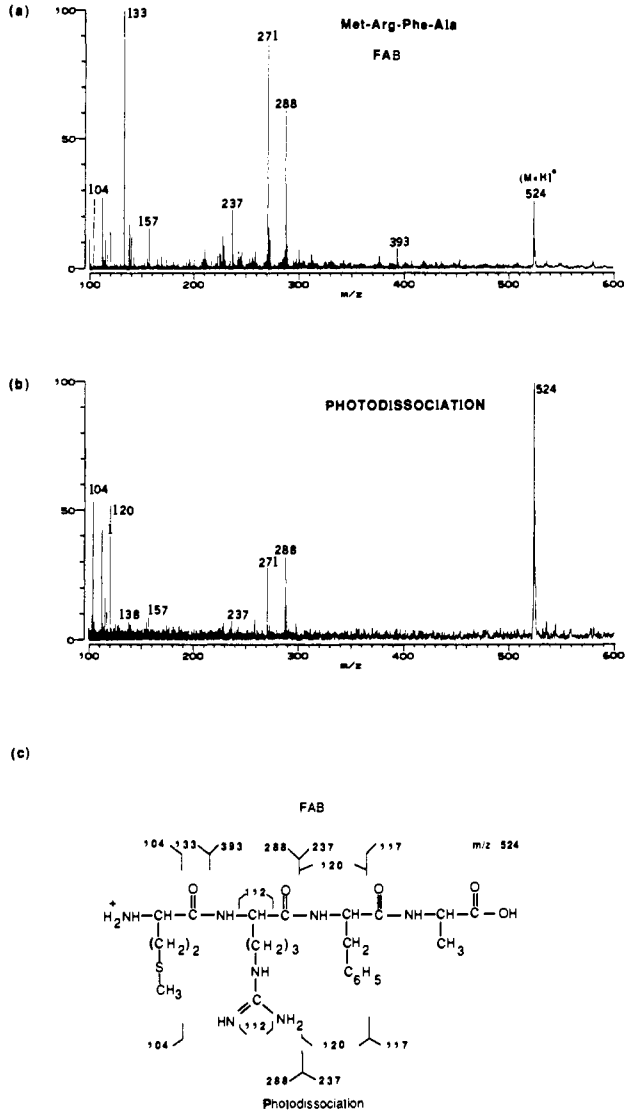

Figure 5. FTMS mass spectra for Met-Arg-Phe-Ala and the peak assignments for the fragment ions: (a) ions produced by 10-keV cesium ion bombardment of the sample dissolved in glycerol, (b) daughter ions produced by photodissociation of m/z 524 with a single laser pulse (wavelength 193 nm and 20 mJ).

Figure 6a is the FAB mass spectrum for gramicidin S obtained with our external ion source FTMS instrument. The peak for the protonated molecular ion, m/z 1141.71, is clearly evident, but the mass resolution is quite low because of insufficient computer memory in our present data acquisition system. Using the broad-band acquisition mode, a FTMS transient of only 16-ms duration can be digitized (2-MHz digitizer rate, 32K data points). The inset in Figure 6a shows, however, that under the same conditions a mass resolution of 120 000 can be obtained and all the isotope peaks can be resolved by using the narrow-band acquisition mode (m/z 1132 to 1164) and a lower digitizer rate (5 kHz). This is an inconvenient characteristic of our present data system, but as computers capable of performing larger FFT calculations become available, it should be possible to obtain a high-resolution FTMS spectrum even over a wide-mass range.

To elucidate the structure of the fragment ion at m/z 571, ejection pulses are used to isolate it and then a single laser pulse (193-nm wavelength, 20 mJ) is fired. Figure 6b shows that the efficiency of the photodissociation process is very high and that a large number of fragment ions are formed. Our proposed structure for the m/z 571 peak, the pentapeptide Leu-Phe-Pro-

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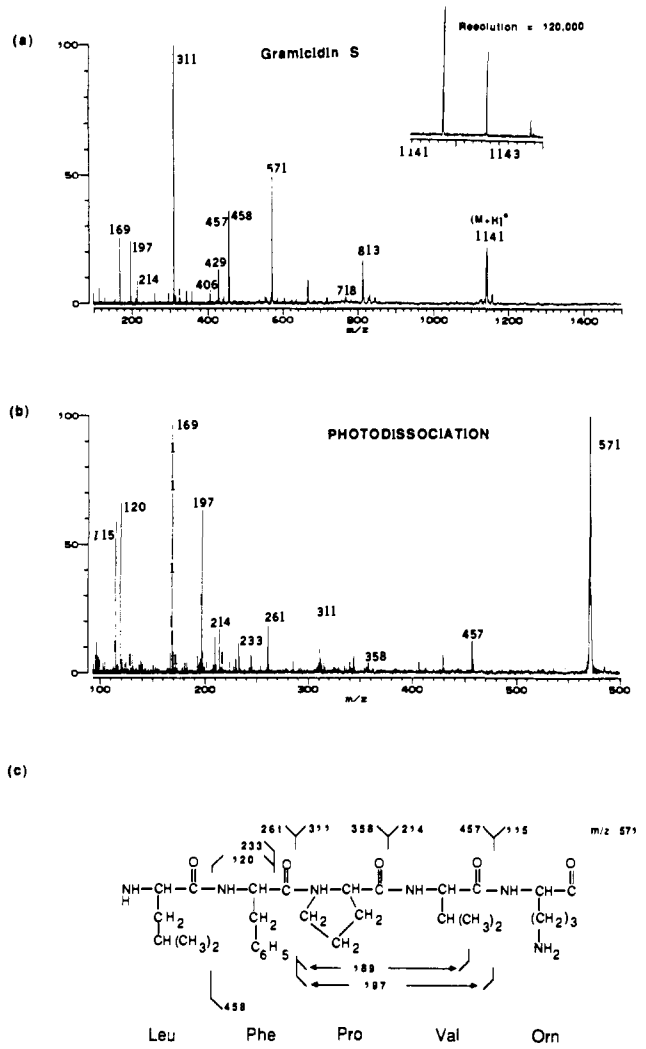

Figure 6. FTMS mass spectra for gramicidin S and the proposed structure for the m/z 571 fragment ion: (a) broad-band and narrow-band (inset) mass spectra gramicidin S dissolved in glycerol and bombarded by 10-keV cesium ions, (b) daughter ions produced by photodissociation of m/z 571 with a single laser pulse (wavelength 193 nm and 20 mJ).

Val-Orn, is shown at the bottom of Figure 6 along with the peak assignments for the photodissociation fragment ions. Table I lists some of the fragment ions that are observed and shows how the measured masses compare with the calculated exact mass for each fragment. Photodissociation works very well in this case. The peaks at m/z 115, 234, 311, and 458 are the Y_1, Y_2, Y_3, and Y_4 sequence ions which retain the C-terminal Orn residue, and the peaks at m/z 261, 358, and 457 are the B_2, B_3, and B_4 sequence ions that retain the N-terminal Leu residue. In addition, Table I shows that all the expected dipeptide and tripeptide fragments resulting from cleavage of the peptide bonds are observed in the photodissociation mass spectrum. An interesting aspect of these results is that gramicidin S is a cyclic decapeptide, and there are, in principle, a multiplicity of possible initial ring cleavages which could produce the 571 fragment ion.[38] Our results indicate, however, that ring cleavage is not random and that the ion formed by FAB results specifically from symmetric cleavage between the Orn and Leu residues on opposite sides of the ring.

Very impressive results reported by Hunt and co-workers showed that Y and B-type ions resulting from peptide bond cleavage also dominate the photodissociation mass spectrum of $(M + H)^+$ ions of a 15 residue tryptic peptide.[35] They also pointed out that the fragmentation observed in laser photodissociation is similar to that seen in collision-induced dissociation experiments.[35,39] Although this is useful for interpreting the structures of peptides, it is intriguing to consider whether more control over the fragmentation might be achieved by varying the laser wavelength and power. For example, it might be possible to activate certain chromophores, such as Phe, His and Trp, and cause a large peptide to fragment preferentially at these linkages. This raises fundamental questions of how quickly the absorbed energy randomizes through the molecule and whether it is possible to cause prompt, photochemical fragmentation as opposed to statistically controlled fragmentation.

Table I. Fragment Ions Produced by Photodissociation of m/z 571 from Gramicidin S[a]

photofragment ions	measd mass	exact mass	rel abund, %
Dipeptide Fragments			
Leu Phe H^+	261.19	261.16	18.9
Leu Phe $H^+ - CO$	233.19	233.17	11.9
Phe Pro H^+	245.16	245.13	7.5
Pro Val H^+	197.14	197.13	64.3
Pro Val $H^+ - CO$	169.14	169.13	98.1
Val Orn H^+	214.17	214.16	17.0
Tripeptide Fragments			
Leu Phe Pro H^+	358.31	358.21	4.3
Phe Pro Val H^+	344.25	344.20	6.8
Pro Val Orn H^+	311.25	311.21	21.3
Tetrapeptide Fragments			
Leu Phe Pro Val H^+	457.39	457.28	13.2
Phe Pro Val Orn H^+	458.39	458.28	5.6
Side-Chain Fragments			
$NHCOCH(CH_2)_3NH_3^+$	115.086	115.087	59.4
$C_6H_5CH_2CH{=}NH_2^+$	120.081	120.081	67.2

[a] The experimentally measured masses and calculated exact masses are given for each fragment.

Earlier we noted that results for protonated Leu-Ala indicated that there is a strong wavelength dependence for photodissociation.[16] Using the ArF excimer laser line at 193 nm gave a good yield of fragment ions, 249 nm was weaker, and no photodissociation was observed with 350-nm light. In this case, the carbonyl group is the chromophore. If the peptide has an aromatic group, such as Phe in our gramicidin S example, the photodissociation cross section at 193 nm is greatly enhanced, most probably due absorption by the Phe chromophore. There does not appear to be any evidence, however, that the Phe residue directs subsequent fragmentation of the molecule. While it is true that Phe gives rise to a large peak at m/z 120, $C_6H_5CH_2CH{=}NH_2^+$, that is not a strong peak in the FAB spectrum; Russell et al. have pointed out that it is a stable and commonly observed ion.[34] For the small peptides that have been studied thus far, it appears that thermodynamic factors, such as site of protonation and stability of the ions formed, are most important in determining the fragmentation mechanism, just as has been observed for FAB fragmentation.[40] For example, the A- and B-type sequence ions result from the high proton affinity of the N-terminal amino acid. And two of the most prominent photofragment ions in Figure 6, m/z 169 and 197, probably result because of the enhanced proton affinity of the secondary amine in proline with respect to the other amino acids.

In many respects, photodissociation has many of the advantages of tandem mass spectrometry for analyzing mixtures. For example, ejecting the background ions and isolating a particular ion in the FTMS analyzer cell accomplishes the same function as the first stage mass spectrometer in a tandem MS/MS experiment. The advantage of this for FAB ionization is that "chemical noise" peaks caused by the glycerol matrix are removed, and all peaks in the photodissociation mass spectrum are known to be derived from the peak that was isolated. In this regard, it is interesting

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to note that even before ion ejection the FTMS spectra seem to have a significantly lower level of low-mass matrix-related background peaks than when the same sample is run on a sector mass spectrometer (VG 7070). One possible reason for this difference is that the time scale of mass analysis by FTMS is many milliseconds compared to only microseconds for a sector mass spectrometer. As a result, matrix-related ions may be less abundant in the FTMS spectra because they undergo metastable decay before they are detected. Another possible explanation is that the pulsed cesium ion beam used in our experiments causes less radiation damage of the glycerol than the continuous FAB beam used with sector instruments.[41]

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X-14885A: An Ionophore Closely Related to Calcimycin (A-23187). NMR, Thermodynamic, and Kinetic Studies of Cation Selectivity

A. M. Albrecht-Gary,*,† S. Blanc-Parasote,† D. W. Boyd,† G. Dauphin,§ G. Jeminet,*,§ J. Juillard,‖ M. Prudhomme,§ and C. Tissier‖

Contribution from the Laboratoire de Chimie Physique et d'Electroanalyse, U.A. 405 CNRS, EHICS, Université Louis Pasteur, 67000 Strasbourg, France, Laboratoire de Chimie Organique Biologique, U.A. 485 CNRS, and Laboratoire des Interactions Solutés-Solvants, U.A. 434 CNRS, Université Blaise Pascal, 63170 Aubière, France. *Received October 20, 1988*

Abstract: X-14885A is an open-chain carboxylic ionophore, recently isolated from a *Streptomyces* strain. NMR data have shown that the free acid form adopts a pseudocyclic conformation in chloroform, while in the neutral A_2Mg complex a rotation of the benzoxazole arm is achieved to accommodate the cation in a sandwich system. The replacement of the -NHMe by an -OH function and of a methyl group by H in position 15 for X-14885A does not lead to a large modification in the conformation of the new microbial metabolite in solution. The acid-base equilibria and the thermodynamics of association with alkali and alkali-earth cations are determined in methanol, from potentiometric and spectrophotometric measurements. Divalent cations M^{2+} form strong complexes of 1:1 (AM^+) and 2:1 (A_2M) stoichiometries, following the sequence $Ca^{2+} > Mg^{2+} > Sr^{2+} > Ba^{2+}$. For $AM^+ + A^- \rightleftarrows A_2M$, $\log K_{A_2M}$ are, respectively, 8.0, 7.3, 6.9, and 6.2 ($I = 0$). A complete kinetic study is carried out in methanol for the A_2Mg and A_2Ca complexes, with use of a stopped-flow system. This work shows that the rate-limiting steps of the formation and dissociation mechanisms are associated with the charged complex AM^+, the addition or loss of the second ligand being faster. As for the closely related ionophore A-23187 (calcimycin), the selectivity calcium/magnesium has predominantly a kinetic origin, with faster formation and dissociation rates of the calcium complexes. The replacement of the -NHMe group in calcimycin by an -OH group in X-14885A has a drastic effect in the acid-base properties of X-14885A and on the acid-catalyzed dissociation pathway of its complexes.

In the growing class of carboxylic polyether ionophores isolated from bacterial strains,[1] A-23187 or calcimycin[2] (Figure 1a) occupies an unusual place as a carrier for divalent cations, especially calcium.[3] Owing to its ability to modify intracellular calcium concentration,[4] it is one of the most frequently quoted chemicals in the biochemical literature. A great number of biological events have been shown to be modified by addition of this ionophore; among them can be cited induction of leucotrienes[5] and interferon[6] production.

The overall transport mechanism in the membrane is an electroneutral exchange[7] of an M^{2+} for $2H^+$, and rate constants for the different steps in the diffusion process have been studied in model phospholipid membranes.[8]

Our general purpose was to understand the formation and dissociation of the neutral lipophilic complex, A_2M (2:1 ligands/cation), which diffuses through the membrane and is responsible for the capture and liberation of the divalent cations at the water/membrane interface. In previous work,[9,10] the thermodynamics of calcimycin complexation by alkali-metal and alkaline-earth cations was studied in methanol or methanol/water systems. Complexation and decomplexation kinetics were also

† Laboratoire de Chimie Physique et d'Electroanalyse, Université Louis Pasteur de Strasbourg.
§ Laboratoire de Chimie Organique Biologique, Université Blaise Pascal.
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