as a moderately intense band, contrary to the observations of Masuda and Miyazawa,[5d] but differs from the Raman frequency of 1296 cm^{-1} [12] and 1294 cm^{-1} reported by Chen and Lord.[6b] Previous infrared studies have reported amide III frequencies of 1280 cm^{-1} [5d] and 1314, 1328 cm^{-1}.[5b] Nevertheless, it is in the range characteristic of α-helical structures.[2b] The peak at 1467 cm^{-1} is tentatively assigned to C-C stretchings of the B_1 species mode occurring in the phenyl ring. The A_1 species mode of C-C stretchings of the phenyl ring is not observed in a FT-IR PA spectrum. The peak at 1171 cm^{-1} can be assigned to the ester, carbonyl stretch.[5b] From Table I, it can be noticed that FT-IR PA and FT-IR polypeptide-KBr pellet frequencies agree reasonably well excepting the amide III frequency. Amide III frequency of 1267 cm^{-1} observed in FT-IR PA spectrum falls in the range generally observed for α-helical structures.[2b] FT-IR PA frequencies of poly(γ-benzyl glutamate) are unabiguously characteristic of α-helical structures. Recently, FT-IR PAS has been successfully applied to several biopolymers[13] and offers itself as a novel method for obtaining infrared spectra by completely eliminating artifactual effects of incorporation into an alkali halide matrix. Its applications to the elucidation of molecular conformation far transcends biopolymer structures and has a general applicability to any chemical system.

Acknowledgment. Our thanks to Dr. K. Krishnan and S. L. Hill of Digilab Inc., Division of Bio-Rad, Cambridge, MA, for their help in the experiments described. V. Renugopalakrishnan thanks Professor M. J. Glimcher for the support and encouragement.

Registry No. Poly(γ-benzyl glutamate) (homopolymer), 25014-27-1; poly(γ-benzyl glutamate) (SRU), 25038-53-3.

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Sequencing of Peptides by Secondary Ion Mass Spectrometry

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Received September 19, 1983

The advent of fast atom bombardment mass spectrometry (FABMS), or liquid SIMS, has added a new emphasis to the analysis of peptides and proteins.[1-3] The pseudo molecular ions from peptides and small proteins with molecular weight over 9000 amu have been observed by FABMS.[4] Although molecular weight information is easily obtainable, the primary sequence of the protein is very difficult to ascertain from the FABMS data due to the low degree of fragmentation and interference peaks from the liquid matrix.[5]

If one employs static secondary ion mass spectrometry (SIMS), in which one or two monolayers of the sample are placed on a surface rather than dissolved in a liquid matrix, the spectral interference from the solvent can be eliminated.[6] For the static SIMS study of most biological molecules, low current density (10^{-9} A/cm^2) primary beam conditions must be employed in order to avoid damaging the sample. With these low beam fluxes the secondary ion flux produced from a given amount of material is often low. Consequently, for many organic molecules, analysis times are long and SIMS has not been used much for their analysis.[6]

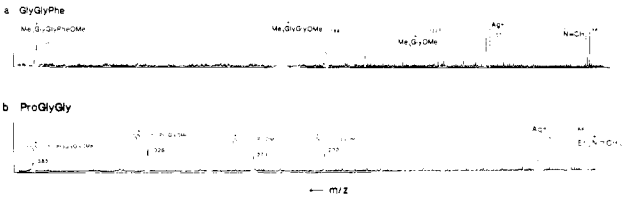
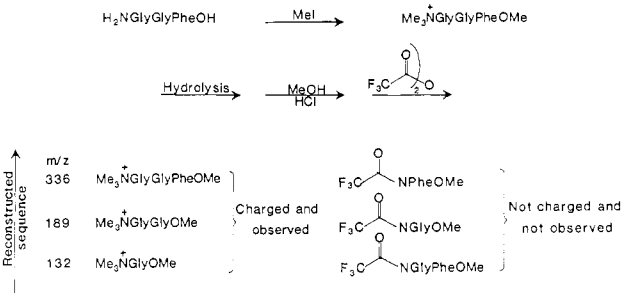


Figure 1. Static SIMS spectra of two tripeptides. (a) Glycylglycylphenyl alanine (GlyGlyPhe) quaternized with methyl iodide, cleaved, esterified, and acylated. Approximately 50 ng of each total hydrolysate were used. Beam conditions: 3 keV Ar^0 $<1 \times 10^{-9}$ A/cm^2; (b) Prolylglycylglycine (ProGlyGly) acylated with chloroacetyl chloride, cleaved, reacted with triethylamine, esterified, and acylated. Beam conditions: 3 keV Xe^0 $<1 \times 10^{-9}$ A/cm^2.

Scheme I



Very recently, Cooks and co-workers[7] have demonstrated that quaternary ammonium salts can be observed at subnanogram levels by SIMS. This detection limit is several orders of magnitude better than for organic molecules that bear no inherent charge. In general, noncharged organic molecules are not readily detected by SIMS and if observed are usually protonated or cationized. This difference in detection limits between charged and uncharged compounds can be used as a basis for the sequencing of peptides.

If the N-terminus of a peptide is labeled with a charged group and is cleaved with acid, esterified, and acylated and the SIMS spectra of the resultant mixture is obtained, then the spectra should preferentially show the ions with the charged group attached. Since these ions originate from only one end, the sequence of the peptide can be readily reconstructed on the basis of the mass differences between the ions. An outline of this sequencing method is shown in Scheme I for the tripeptide GlyGlyPhe. Figure 1 depicts the static SIMS spectra, from a silver surface, of two tripeptides labeled by two different methods at the N-terminus.

The sequence of the peptides are clearly evident from the spectra with no interferences from the uncharged materials in the matrix. (It should be emphasized that the static SIMS spectra were taken of unpurified mixtures.) The nonsequence ions at m/z 107/109, 86, and 58 in Figure 1 part a and/or b, correspond to Ag^+ ions from the substrate and amine fragment ions of the derivatizing reagents. The ion at m/z 129 in Figure 1a is attributed to an unknown impurity, but its presence causes no trouble in determining the sequence of the peptide since its mass is lower than that of the glycine derivative. Proline-containing peptides are known to form diketopiperzines.[8] If a diketopiperzine is produced, this would release free glycine, which will be derivatized and observed in Figure 1b at m/z 232.

Quaternization with methyl iodide requires severe conditions and usually gives poor yields of the quaternary ammonium de-

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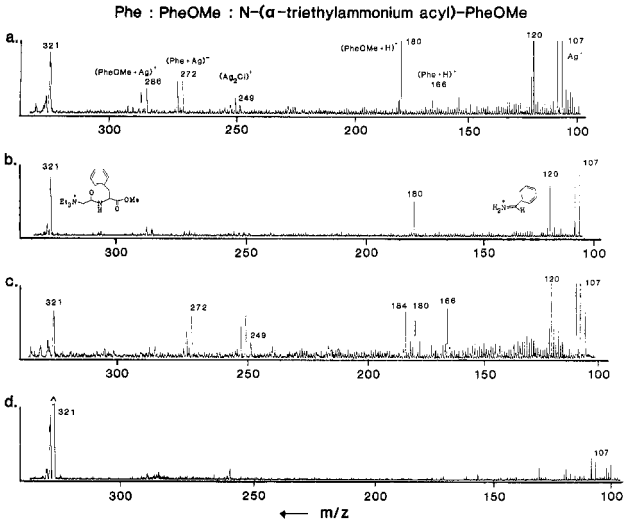

Figure 2. Static SIMS spectra of an equal molar mixture of phenylalanine (Phe), phenylalanine methyl ester (PheOMe), and *N*-((triethylammonio)acetyl)phenylalanine methyl ester at various pH's. Spectra are recorded from acid-etched silver surfaces using 3-keV Xe^0 2×10^{-9} A/cm^2. Spectrum a was obtained at a slightly acidic pH, b was obtained at a more basic pH, c was obtained at pH <2, and d was the charged acyl derivative alone.

rivative.[9] A better approach to labeling the N-terminus is by acylation with chloroacetyl chloride. This is best accomplished by heating the peptide and the reagent in an inert solvent[10] or by the Scotten-Baumann technique if the peptide is water soluble.[11] After acylation, the peptide is cleaved by acid, esterified with 3 M HCl in methanol, and treated with triethylamine to form the ammonium salt.[12]

These derivatization reactions have two principal objectives: (1) to label the peptide with a charged group and (2) to eliminate any potential sites for charge production by protonation of components in the mixture. This second objective is accomplished by esterification and acylation reactions. If these precautions are not taken, the static SIMS spectrum of all cleavage products would be observed, depending on the pH. As an example, the SIMS spectra of an equal molar mixture of phenylalanine, phenylalanine methyl ester, and *N*-((triethylammonio)acetyl)phenylalanine methyl ester deposited on a silver surface are shown in Figure 2. Spectrum 2a was obtained when the mixture was deposited at a slightly acidic pH. At this pH, the phenylalanine is in the zwitter ionic form and has no net charge. Consequently, ions from it are observed at only low intensity compared to the protonated methyl ester and the charged acyl derivative. Spectrum 2b shows the same mixture deposited at a more basic pH. No phenylalanine molecular ions are present and the methyl ester molecular ions are reduced in intensity. Spectrum 2c was taken at pH <2 where ions from all three components are observed. In all three spectra, silver-cationized ions of phenylalanine and its methyl ester, but not the charged acyl derivative, are also observed. The charged acyl derivative is seemingly insensitive to the chemical environment from which it is deposited and when examined alone (as in the spectrum in Figure 2d) shows little, if any, fragmentation.

The derivatization methods suffer unfortunately from being nonspecific. Any basic internal amino acid would also be derivatized, and these extra ions would complicate the mass spectrum. This problem can be overcome by protecting any internal lysines as thioureas with one cycle of an Edman degradation and then performing the next cycle with a charged isocyanate rather than an isothiocyanate. Unfortunately, the commercially available 2-chloroethyl isocyanate undergoes ring formation[13] in triethylamine solution faster than formation of the ammonium salt. Attempts to prepare the ammonium salt of the isocyanate before reaction with the peptide were also unsuccessful. Derivatization with other charged isocyanates is in progress and will be reported shortly.

Although FABMS has been used to examine the peptide derivatives, static SIMS gives a better signal-to-noise ratio and lower intensities of matrix ions. Also, the relative signal intensities of the ions in the static SIMS spectra are more representative of the components in the sample than with FABMS. For example, the FABMS spectra of equal molar mixtures of similar quaternary salts can show very different intensities for the molecular ions[14] due to the different ways the molecules reside on the surface of the liquid matrix.

In summary, we have developed a general peptide sequencing technique that relies upon the enhanced detection of charged groups in SIMS or FABMS. The peptide may be sequenced from the *n*-terminus. The current level of detection is in the low nanogram range.

Acknowledgment. D.A.K. and M.M.R. thank the National Research Council for Resident Research Associateships and Maria B. Barnes for preparing some of the figures.

Registry No. Me_3^+GlyGlyPheOMe, 89178-06-3; Et_3^+-NCH_2COProGlyGhyOMe, 89196-36-1; phenylalanine, 63-91-2; phenylalanine methyl ester, 2577-90-4; *N*-(triethylammonio)acetylphenylalanine methyl ester, 89178-07-4.

(9) Typical conditions for quaternization with methyl iodide: 1 mg of the peptide is dissolved in 2 mL of 50% methanol/water; a 10-fold excess of methyl iodide is added in portions, while keeping the pH at 10 using lithium or potassium hydroxide in methanol as base.
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Activation of Methane by Supported Rhodium Complexes

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Received April 25, 1983

Alkane "activation" is a current theme in organometallic chemistry that commands considerable attention derived in part from interest in selective catalytic conversion of alkanes to organic products. In this context, two disparate approaches can be considered for developing catalysts for selective alkane activation: These involve "electron-deficient" species or "electron-rich" ones. In the former category fall alkane isomerization catalysts and other species associated with conversion of alkanes to products via carbonium ions.[1] The latter category includes complexes of metals such as those of Ir(I) containing strong donor ligands which "oxidatively add" simple alkanes stoichiometrically.[2] Of these two approaches, the former one seems most auspicious for seeking *catalytic* systems for alkane activation: many of the most interesting of these processes will be oxidative in nature, and reagents to accomplish these transformations likely would be incompatible with strongly reduced transition-metal centers. Our recent investigations concerning supported rhodium complexes show that it is possible to use an oxide to help stabilize the metal in a high

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