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Chromatography/SIMS: Applications to Peptides and Bile Acids

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Introduction

Biological molecules are often analyzed by fast atom bombardment (FAB) and secondary ion mass spectrometry (SIMS), both of which are "soft" ionization techniques that produce abundant ions containing molecular weight information. Fragment ions are of low abundance relative to the molecular ions, and, in the case of peptides, such ions can be interpreted to provide the amino acid sequence. The biological molecules analyzed by FAB and SIMS are incompatible with separation by gas chromatography. Liquid chromatography can be used, but the interface to mass spectrometry is not yet perfected. We have constructed a chromatography/SIMS instrument that directs a beam of primary ions onto a thin-layer chromatogram positioned inside the instrument (3), and have used this instrument for the analysis of mixtures of peptides and bile acids. Secondary ions are sputtered from the intact thin-layer chromatogram with the aid of a phase-transition matrix (2), and are similar in appearance to the mass spectra of the pure samples in glycerol. In addition to the molecular weight and structural information, the x and y spatial profile of the ions in the mass spectrum can be measured, providing an organic ion image of the surface. A complete mass spectrum can also be obtained at each point in the chromatogram. This SIMS detection system offers unique advantages in comparison to conventional methods of TLC analysis and other bioanalytical techniques such as GC/MS. These advantages include: 1) independent access order to sample spots, 2) variable spatial resolution, 3) preservation of chromatogram integrity, 4) variable integration time, and 5) four-dimensional data: x, y in distance, mass-to-charge ratio, and ion intensity or relative abundance.

Experimental

SIMS mass spectra were obtained by dissolving approximately 1 mg sample into 0.3 mL glycerol or a molten mixture of sorbitol and p-toluene sulfonic acid. Spectra were obtained using a modified Extranuclear mass spectrometer equipped with a commercial cesium ion gun (Phrasor Scientific). TLC/SIMS data was obtained by depositing 100 ug sample onto a silica gel (0.2 mm) thin layer chromatogram. The ion source used to obtained the three-dimensional image was a liquid gallium ion gun manufactured by FEI.

Leucine enkephalin and methionine enkephalin were obtained from Sigma Chemical Co. Cholic acid, chenodeoxycholic acid, lithocholic acid and various solvents used were obtained from Aldrich Chemical Co. All were used without further purification.

Results and Discussion

The SIMS mass spectra of the bile acids studied show no protonated molecules; instead loss of H_2O occurs readily. The SIMS spectrum of pure cholic acid, the trihydroxy bile acid, shows as the base peak the $(M + H - 3H_2O)^+$ ion (m/z 355) and another prominent ion at m/z 373 which corresponds to $(M + H - 2H_2O)^+$. Chenodeoxycholic acid, the dihydroxy species, shows one ion at m/z 357 corresponding to $(M + H - 2H_2O)^+$. Lithocholic acid, the monohydroxy species, shows only ions that correspond to the incorporation of sodium ions. Thus, to obtain this spectrum, the glycerol matrix was doped

with NaCl. The base peak is m/z 421, $(M + 2Na - H)^+$. The three-dimensional image of the three bile acids separated on a thin-layer chromatogram was then obtained (Figure 1). By moving the platform in successive steps in x and maintaining a constant y, and

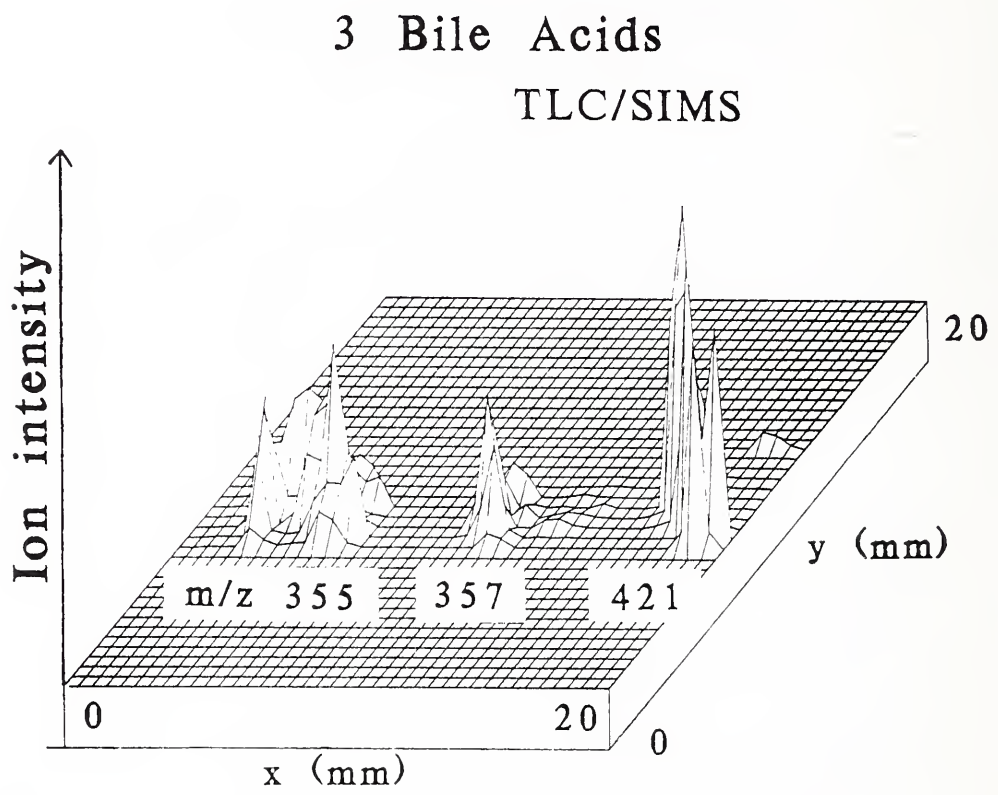

FIGURE 1. Three-dimensional image of the cholic acid (left), chenodeoxycholic acid (center) and lithocholic acid on a silica thin layer chromatogram.

in monitoring the appropriate ion for each acid, the spatially-resolved image was measured. The procedure was repeated changing the y value until an entire image was obtained. The figure shows that the spots are completely resolved, and signal-to-noise ratios are excellent. In addition, the size of each chromatographic spot can be determined by inspection. As mentioned above, data is generated in four dimensions: x, y, m/z, and ion intensity.

FAB and SIMS mass spectra of peptides typically contain the protonated molecule of the peptide and fragment ions that correspond to sequential cleavages at the peptide bonds (1). In this way, the amino acid sequence of a given peptide can be deduced from the masses of the fragment ions. Figure 2 is the SIMS spectrum of the pentapeptide leucine enkephalin taken directly from a TLC plate. The protonated molecule is seen at m/z 556, and the $(M + Na)^+$ is seen at m/z 578. Sodium is an impurity often present in TLC plates. A prominent fragment ion is observed at m/z 278, and the ion labeled 303 and the other, smaller ions seen are either system contaminants or matrix ions. Methionine enkephalin (Tyr-Gly-Gly-Phe-Met), also shows a protonated molecular ion at m/z 574 and a fragment ion at m/z 278, which is the same ion as that in the spectrum of leucine enkephalin; it corresponds to loss of the 2 C-terminal amino acids leaving $(Tyr\text{-}Gly\text{-}Gly + H)^+$. A three-dimensional image of the 2 enkephalins separated on a TLC plate was obtained by monitoring this fragment ion, as shown in Figure 3.

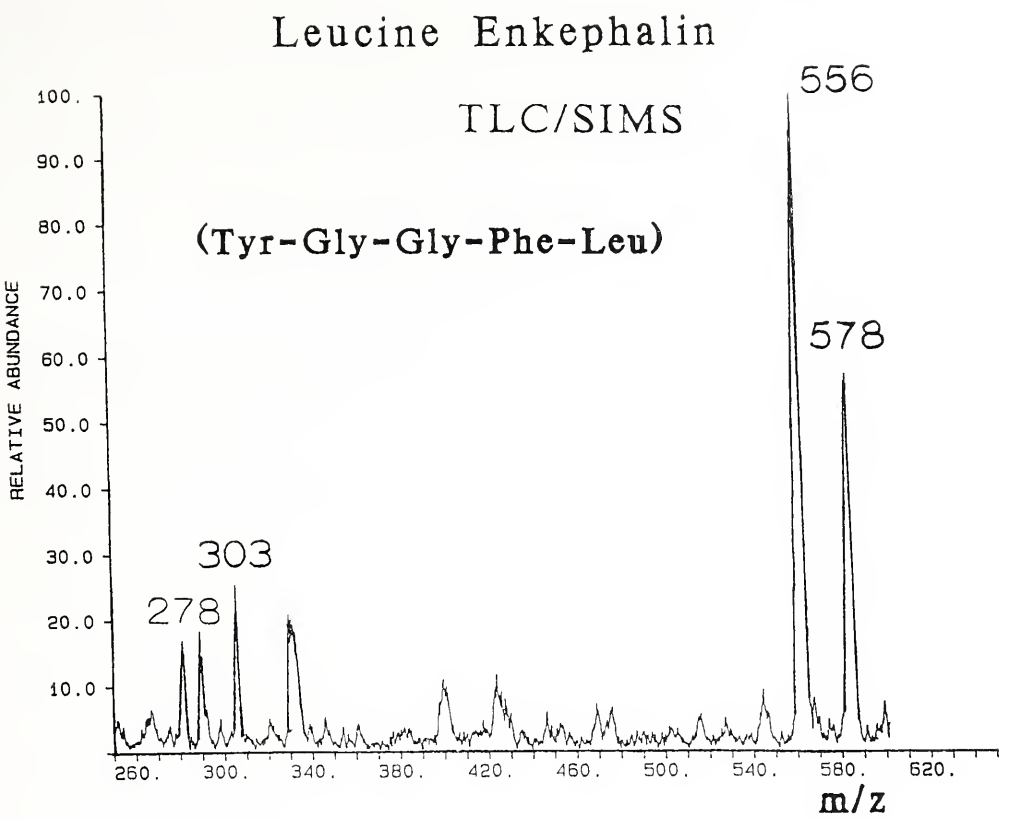

FIGURE 2. TLC/SIMS spectrum of leucine enkephalin.

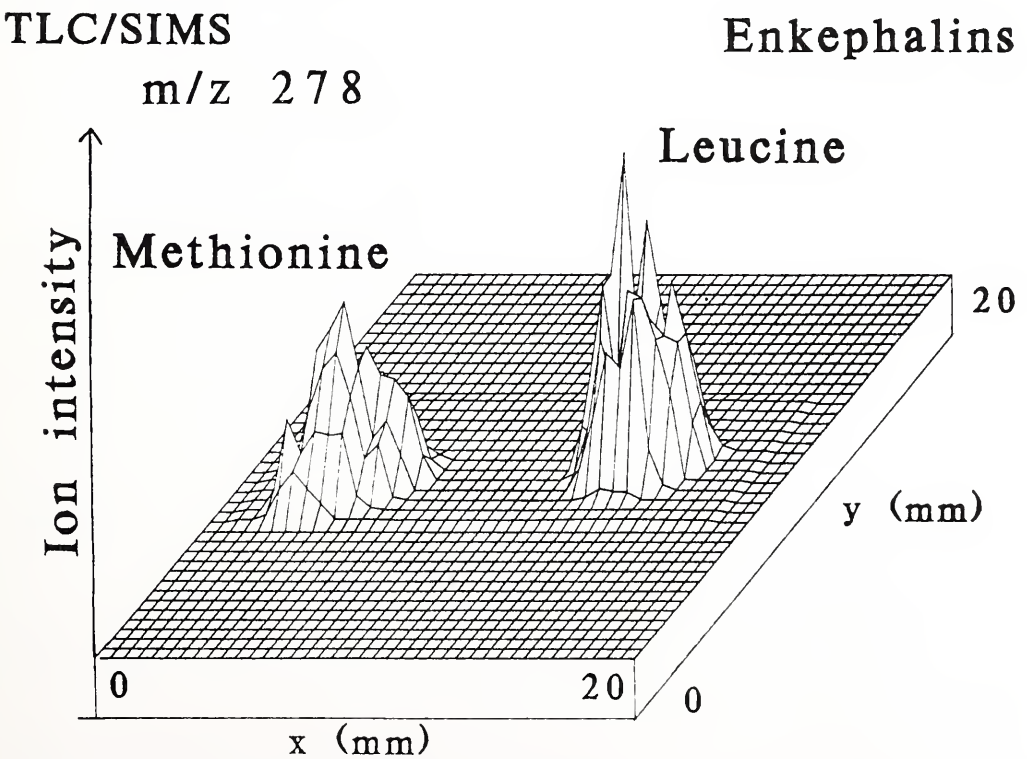

FIGURE 3. Three-dimensional image of leucine and methionine enkephalin on a silica thin layer chromatogram.

This figure illustrates an application of SIMS termed "spatially resolved selected sequence monitoring" of peptides. As shown, each peptide that dissociates to form an ion of specified sequence, selected by mass, will produce a local maximum in ion abundance in the three-dimensional plot. Peptides that are incompletely separated by the chromatography, or even those that exceed the mass range of the mass spectrometer but that still fragment to produce an ion of that specified sequence, can be identified. Thus, enkephalin derivatives would be distinguished from other peptides by monitoring the ion at m/z 278. Experiments continue in the development of this unique application.

Conclusions

Chromatography/SIMS is a viable technique for the analysis of biomolecules. Three-dimensional organic images of the compounds separated by TLC can be generated without substance elution. Chromatogram integrity is maintained, and repetitive analyses can be completed, making feasible important new experiments such as the spatially resolved selected sequence monitoring.

Literature Cited

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