Anal. Chem. **2002,** *74,* 2228−2231

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Infrared Laser Desorption/Ionization on Silicon

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Laser desorption/ionization from a single-crystal silicon surface was performed using a laser operating in the 3-μm region of the mid-infrared. Analyte molecules up to 6 kDa were ionized with no added matrix. As with ultraviolet desorption/ionization from porous silicon (DIOS), IR laser desorption from silicon does not produce matrix ions that can interfere with analysis of low-mass analytes. However, in contrast to UV DIOS, silicon porosity or roughness is not required for ionization using an IR laser. Mass spectra were obtained in the wavelength range between 2.8 and 3.5 μm, which is consistent with energy absorption by a hydrogen-bonded OH group. A mechanism based on desorption of adsorbed solvent molecules is postulated.

Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry is now a standard technique for the analysis of biological macromolecules.[1] The advantages of MALDI include high sensitivity, excellent mass resolution and accuracy, and a wide mass range. The technique is particularly useful for proteomics applications and has become one of the primary analysis methods for protein identification following 2-D gel electrophoresis separations.[2] In a typical MALDI analysis, the biomolecule of interest is desorbed from a metal surface in an excess of an organic acid matrix using a 337-nm ultraviolet laser. Selection of suitable matrixes and optimization of sample preparation methods are areas of ongoing research.[3] Areas of interest include production of homogeneous targets, using small sample quantities, and elimination of interferences, particularly those due to matrix ions in the low-mass region.

It was recently shown that a porous silicon surface can act as a matrix for desorption and ionization of small molecules.[4] This new technique is called desorption/ionization on porous silicon (DIOS), and it shows much promise due to its high sensitivity, tolerance for impurities, and particularly for the lack of low-mass interference due to matrix ions. The latter quality makes DIOS particularly well suited to studies of molecules below ∼700 Da that are otherwise difficult to observe with laser desorption/ionization. DIOS has been demonstrated in proteomics[5] and forensics[6] applications and has been used for the direct detection of peptides from cells cultured on the silicon surface.[7] Several studies have been undertaken to adduce the mechanism of DIOS, and the initial results indicate that silicon porosity or roughness is necessary for successful desorption and ionization.[8−11] Porous silicon surfaces can be formed by electrochemical etching,[8] chemical vapor deposition,[11] or spark etching.[12]A hyperthermal atomic beam technique has been used to etch silicon to produce roughened surfaces for laser desorption.[10] Postetching treatment of porous silicon surfaces was found to be important to maintain their performance. Storage of etched wafers in an ethanol solution gives the best performance over a long period of time,[8] and other studies have suggested a correlation between surface wetting and DIOS performance.[9] These studies suggest that residual solvent adsorbed on the porous silicon surface may be integral to the DIOS process.

It is known that the protic solvents glycerol,[13] water,[14] ethanol,[15] and methanol[15] function as MALDI matrixes when a mid-IR laser is used to excite the OH stretch vibrational mode. Water is a particularly notable IR MALDI matrix because it functions best under nominally matrix-free conditions: IR MALDI can be performed at 2.94 μm using lyophilized proteins, air-dried protein solutions, or protein crystals without the addition of matrix as long as the sample is frozen to prevent the loss of waters of hydration in vacuum.[14] In the work described below, desorption and ionization from silicon was performed using a tunable mid-IR laser

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10.1021/ac0112972 CCC: $22.00
Published on Web 03/29/2002

in order to investigate solvent effects in desorption and ionization of molecules from silicon surfaces.

EXPERIMENTAL SECTION

The MALDI TOF mass spectrometer used in this study was described in detail previously.[15,16] Briefly, the instrument is a 1-m linear TOF with delayed ion extraction and a dual-microchannel plate detector. Samples can be attached directly to a cryogenically cooled sample stage or inserted into the mass spectrometer through a vacuum insertion lock. For the work described below, the sample stage was operated at room temperature and the vacuum insertion lock was used. The IR source is a Nd:YAG pumped optical parametric oscillator (OPO) that is tunable from 1.45 to 4.0 μm. The OPO output is focused onto the sample to a spot size of approximately 150 $\times$ 200 μm as determined using laser burn paper and a measuring magnifier. At this spot size, the OPO irradiance used to obtain the mass spectra was ~60 MW/cm^2 and this corresponds to a fluence of 0.3 J/cm^2. Each mass spectrum below was obtained as an average of 10 single-shot spectra. It was found that a relatively low extraction field improves the ion signal for IR MALDI.[17,18] In the current apparatus, the target to acceleration lens spacing is 19 mm and the extraction field strength is 150 V/mm. Under these conditions, the optimum delay between laser irradiation of the sample and ion extraction is typically between 1 and 6 μs.

A 0.6-mm-thick antimony-doped single-crystal silicon (100) wafer (Silicon Quest International, Santa Clara, CA) with resistivity between 0.40 and 0.48 was used as the sample target. The surface roughness of the polished silicon wafers is on the order of 1 nm. The wafer was scored and broken into 4-mm squares that were affixed to the stainless steel sample probe with conductive double-sided tape (Electron Microscopy Sciences, Ft. Washington, PA). No etching or roughening of the silicon surface was performed unless otherwise indicated. The analytes bradykinin (B-3259 Sigma, St. Louis, MO), gramicidin D (G-5002, Sigma), bovine insulin (I5500, Sigma), riboflavin (R-9504, Sigma), and L-ascorbic acid (25,556-4, Aldrich, Milwaukee, WI) were used as obtained from the manufacturer. For initial studies, analyte solutions were made by dissolving the analyte in pure ethanol (200 Proof, AAPER Alcohol, Shelbyville, KY) at a concentration of 100 μM. For lower concentrations, a 3:1 (v/v) mixture of methanol or ethanol and water was found to be more effective and was used to obtain spectra at concentrations down to 10 nM. For the spectra shown below, a 3-μL deposit on the wafer was allowed to dry to form a sample spot of ~2 mm in diameter.

RESULTS AND DISCUSSION

A mass spectrum of the peptide bradykinin obtained by irradiating an untreated silicon target with 2.94-μm laser radiation is shown in Figure 1a. Bradykinin was used as a test analyte because it is a readily obtained MALDI mass standard that can give a good indication of the relative utility of various procedures. The base peak in the mass spectrum is protonated bradykinin, and there are sodium ([M + Na]$^+$) and potassium ([M + K]$^+$) cation adduct peaks roughly 20−30% as intense as the [M + H]$^+$ base peak. The mass resolution is 400 fwhm, comparable to that observed with an organic acid MALDI matrix. The extraction delay was 2.2 μs. A peak at m/z = 904 corresponds to the protonated y_8 fragment of bradykinin (and the associated Na$^+$ and K$^+$ adducts) that results from in-source dissociation prior to extraction.[19] The mass spectrum in Figure 1b is a mass spectrum of bradykinin deposited on a stainless steel target with all other conditions the same as for Figure 1a. Peaks in the low-mass region correspond to sodium and potassium cations and what is apparently diffusion pump oil; these peaks are considerably larger than the protonated analyte peak. The ionization mode for the bradykinin deposited on the metal target may be laser desorption ionization (LDI) or possibly MALDI with residual water or solvent in the sample acting as the matrix.[14] The mass spectra in Figure 1 suggest a silicon surface enhancement similar to UV DIOS, but it is also possible that the difference in signal intensity between the silicon and stainless steel substrates results from different analyte and surface interactions, crystallization conditions, or substrate molar absorptivity and thermal conductivity.

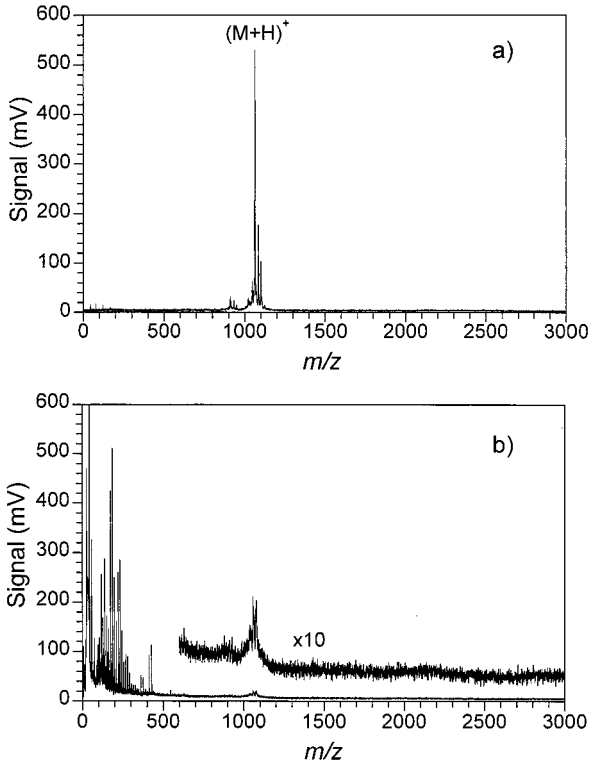

Figure 1. Laser desorption/ionization mass spectra of bradykinin at 2.94 μm from (a) silicon and (b) stainless steel targets.

Bradykinin mass spectra could be obtained between 2.8 and 3.5 μm with an abrupt loss of signal when the laser was tuned to shorter wavelength and a more gradual loss of signal when the laser was tuned to longer wavelengths. These results are similar to those observed with solid-matrix IR MALDI.[21] The wavelength range roughly matches the OH stretch absorption of solid and

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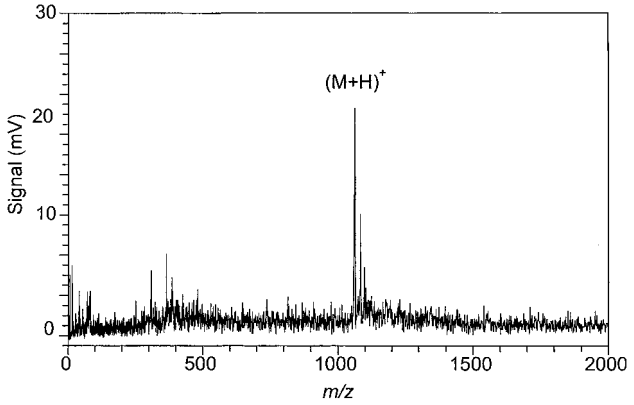

Figure 2. Silicon target laser desorption/ionization mass spectrum of 30 fmol of bradykinin at a wavelength of 2.94 μm.

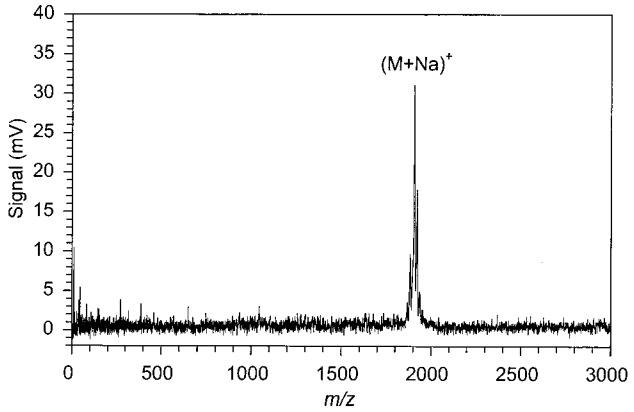

Figure 3. IR laser desorption ionization of gramicidin D powder deposited on silicon.

liquid IR MALDI matrixes[18,20−22] and also corresponds closely to the IR absorption spectrum of a surface film of water on silicon.[23]

The detection limit for IR desorption from silicon is similar to that observed when the mass spectrometer is used with IR or UV MALDI. Figure 2 shows a mass spectrum of bradykinin that was obtained using a 30-fmol quantity of analyte deposited on the silicon surface from a 10 nM mixture of ethanol and water 3:1 (v/v) with 0.1% TFA. Mixtures of either methanol or ethanol and water were found to give the best performance for analytes at low concentrations. The base peak in the spectrum is associated with protonated bradykinin, and sodium and potassium adduct peaks of bradykinin are also observed. The peaks in the low-mass region result from surface contamination, possibly from vacuum pump oil.

It was found that mass spectra could be obtained from silicon surfaces under a variety of conditions. Mass spectra obtained from the unpolished surface of the silicon wafer and from the polished surface roughened with No. 400 sandpaper are indistinguishable from those obtained from the polished surface (data not shown). It was also discovered that mass spectra could be obtained simply by sprinkling dry analyte powder from a spatula onto the silicon surface. A mass spectrum of gramicidin D peptides obtained with dry powder deposition is shown in Figure 3. The mass resolution

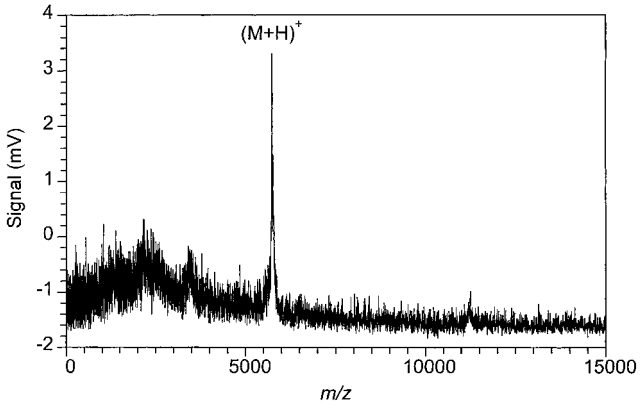

Figure 4. IR laser desorption/ionization of bovine insulin deposited on silicon from a 3:1 (v/v) solution of water and methanol.

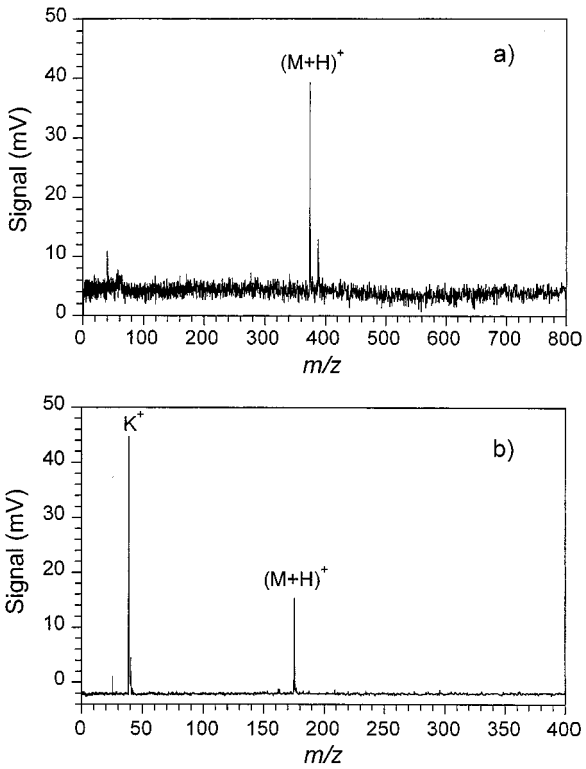

Figure 5. Silicon target infrared laser desorption/ionization mass spectra of (a) riboflavin and (b) ascorbic acid.

is not sufficient to separate the mixture of four peptides and their associated protonated and alkali cation adduct ions, but the spectrum exhibits the characteristic lack of interference ions of desorption from silicon.

The largest analyte ion that was observed in this study was bovine insulin (M_r = 5733.6). A mass spectrum of bovine insulin using IR laser desorption from silicon is shown in Figure 4. Here, the analyte was dissolved in a 3:1 (v/v) mixture of water and methanol to a concentration to 100 μM and the desorption wavelength was 2.94 μm. An extraction delay of 6 μs was found to result in the optimum analyte signal. Some fragmentation is observed, which may be a result of the relatively long extraction delay time. Mass spectra of small-molecule analytes are shown in Figure 5. Figure 5a depicts a mass spectrum of riboflavin, and Figure 5b is a mass spectrum of ascorbic acid. The spectra are characterized by large protonated molecule peaks and no interfer-

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ence from matrix ions. The large potassium cation peak in the caffeine mass spectrum is attributed to an impurity in the sample.

The observations that surface porosity is not required for enhanced IR LDI from a silicon surface and that the wavelength range for this enhancement is similar to that of hydrogen-bonded surface species suggest that the matrix, taken here as the material that absorbs the laser energy and promotes desorption and ionization, is endogenous water or solvent molecules at the silicon surface. We postulate that the IR laser excites the solvent OH stretch, leading to desorption and ionization of surface solvent and analyte followed by ionization in the expanding plume. With UV DIOS, energy from a UV laser is absorbed by the silicon itself, which may then transfer the energy to the adsorbed solvent molecules. In the UV case, the porous silicon surface may be necessary for efficient transfer of energy from the silicon to the adsorbed solvent. According to this interpretation, the UV DIOS process is similar to that of the two-phase matrix consisting of finely divided particles in a liquid suspension, as was suggested previously by Alimpiev et al.[10] Experiments aimed at testing this interpretation are currently underway.

CONCLUSIONS

We have demonstrated enhanced laser desorption/ionization from untreated silicon surfaces using a mid-IR laser. Because the technique is exogenous matrix-free, there is no interference in the low-mass region that is due to matrix ions. The performance of silicon surface IR LDI is comparable to that reported for UV DIOS, with the exception that the tolerance for salt does not appear to be as great.[2] The observed detection limit of a few tens of femtomoles is a factor of 10 greater than that reported for UV DIOS[4] but is comparable to the detection limit observed for UV MALDI with our instrument.

The potential applications for IR LDI on silicon include those reported and suggested for UV DIOS, including small-molecule mass spectrometry and integration with derivatized surfaces and lab-on-a-chip technology. In addition, because silicon is transparent in the IR, illumination of the sample from the back in transmission geometry might be used.[24] This configuration may be useful for spatially resolved analysis of samples such as tissue or deposited aerosol particles since it allows the use of complex focusing optics that might not otherwise be compatible with the ion extraction optics.

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ACKNOWLEDGMENT

This work is supported by the National Institutes of Health Grant R42RR15134 and by the National Science Foundation Grant CHE-0196568.

Received for review December 31, 2001. Accepted March 8, 2002.

AC0112972