

Soft Laser Desorption/Ionization Mass Spectrometry Using a Pyroelectric Ceramic Plate

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A novel matrix-free soft laser desorption/ionization mass spectrometry using a pyroelectric ceramic plate with a flat surface as an ionization platform was developed. Molecular-related ions of thermally unstable compounds such as peptides, oligosaccharides, and surfactants could be observed on the mass spectra with almost no fragmentation of the analytes.

Among soft laser desorption/ionization mass spectrometry (SLD-MS) techniques, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) has attained an important position in the field of biological analysis and polymer characterization. However, the use of matrix reagents in MALDI-MS frequently poses serious problems in the characterization of lower molecular weight compounds. Specifically, the strong background peaks originating from the matrix reagent interfere with MALDI mass spectra in the lower mass range (typically under m/z 500, but sometimes over several thousand). In order to avoid matrix ion interference, some nonvolatile inorganic matrices such as cobalt powder,¹ TiO₂ particles,² C₆₀,³ and carbon nanotubes⁴ have been proposed. The use of fine inorganic particles, however, frequently causes the pollution inside the ionization chamber of the MS instrument by scattering the particles during the measurements with UV laser radiation.

Recently, a matrix-free SLD-MS technique using porous silicon as an ionization platform, referred to as "desorption/ionization on porous silicon-mass spectrometry" (DIOS-MS), was proposed.⁵ More recently, another matrix-free SLD-MS using silicon nanowire deposited on silicon-wafers has also been reported.⁶ In these techniques, molecular-related ions are generated on the silicon-wafers having submicron-sized structures on which the analytes are deposited. Another work has been reported that an anatase-type nanocrystalline TiO₂ having mesoporous morphology formed through a sol-gel deposition can be act as an ionization platform.⁷ Although the ionization mechanisms of these SLD-MS are poorly understood, most of the researchers in this field have speculated that submicron-sized structures formed on the silicon-wafers^{5,6} or deposited nanocrystalline TiO₂⁷ might act as a matrix based on the efficient UV laser absorbance.

A typical pyroelectric ceramics, lead lanthanum zirconate titanate (PLZT), is a transparent for visible light but UV-absorbing material, with UV radiation generating strong electric fields on the crystal surface. Therefore, we have speculated that the pyroelectric nature of PLZT might assist soft laser desorption/ionization of thermally unstable compounds. In this letter, we report that a flat PLZT plate with no submicron-sized structures enable to be an ionization platform for matrix-free SLD-MS.

The PLZT plate [(Pb_{1-x}La_x(Ti_yZr_z)O₃), $x/y/z = 0.09/$

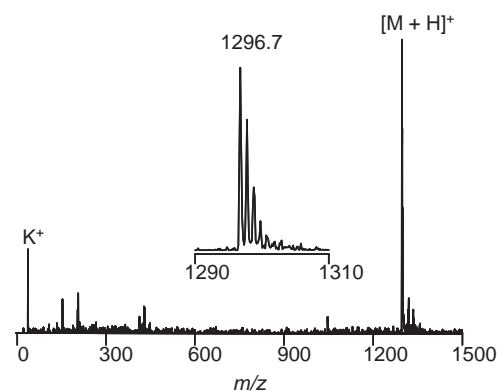


Figure 1. SLD mass spectrum of angiotensin-I.

0.65/0.35; size, 5 × 5 mm; thickness, 0.2 mm; both-surface-polished] used in this study was purchased from Furuuchi Chemical Co. (Tokyo, Japan). The PLZT plate has essentially flat surface of which roughness is less than a few nanometer announced by the manufacture. The PLZT plate was mounted onto a standard MALDI stage using electroconductive adhesive tape. A broad range of samples involving biomolecules and industrially produced compounds were used. Angiotensin-I, which is usually used as a standard peptide to evaluate the performance of MALDI-MS instruments, was dissolved in a mixture of methanol and deionized water (30/70 v/v) containing 0.5% trifluoroacetic acid at a concentration of ca. 200 pmol/μL. For oligosaccharide samples, equal amounts of α- and β-cyclodextrins (CD) were dissolved in methanol (0.5 mg/mL each). Octylphenol polyethoxylate, which is a typical nonionic surfactant commercially known as Triton-X 100, was used as a model sample of industrially produced compounds. The surfactant sample was dissolved in methanol at a concentration of 1 mg/mL. For the ionization of the oligosaccharide and surfactant samples, NaI (1 mg/mL in acetone) as a cationization salt was mixed with the sample solutions at a ratio of 1/5 (v/v) to form the sodium adduct ions ([M + Na]⁺) of the analytes.

Each sample solution (ca. 1.0 μL) was spotted onto the PLZT plate and dried in air. The SLD-MS measurements were performed in basically the same way as in general MALDI-MS measurements using a Voyager DE-PRO time-of-flight mass spectrometer (Applied Biosystems, Framingham, MA, USA) equipped with a pulsed nitrogen laser ($\lambda = 337$ nm, 3 ns pulse width, and a 3 Hz frequency) and a delayed extraction ion source. Ions generated by laser desorption were introduced into the flight tube at an acceleration voltage of 20 kV for the reflector (2.0 m flight path) positive ion mode. The laser beam intensity was set to just above the threshold for analyte ionization.

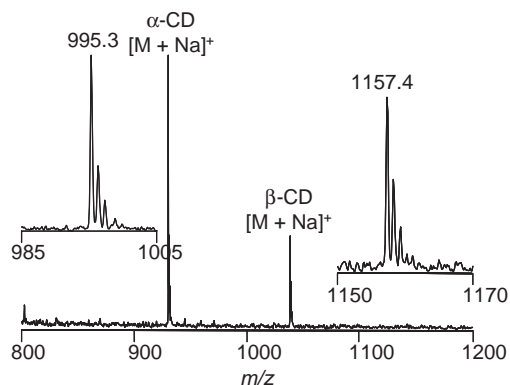


Figure 2. SLD mass spectrum of the mixture of α - and β -CD.

Figure 1 shows the SLD mass spectrum of angiotensin-I. A monoisotope peak of the protonated molecule ($[M + H]^+$) was observed at m/z 1296.7. Here, the weak peaks observed in the lower mass region could mainly be attributed to impurities such as potassium and plasticizers. This observation suggests that the soft laser desorption/ionization of angiotensin-I with almost no fragmentation occurred on the PLZT surface.

Figure 2 shows the SLD mass spectrum of the mixture of α -CD and β -CD observed in the presence of NaI as the cationization salt. Strong monoisotope peaks of the sodium adduct ions ($[M + Na]^+$) were observed at m/z 995.3 for α -CD and m/z 1157.4 for β -CD, respectively. Since oligosaccharides are thermally unstable compounds, fragmentation mainly based on dehydration could easily occur when excess energy was added to the sample molecules during desorption/ionization. The fact that no dehydrated fragment peak was observed indicated that thermally unstable oligosaccharides could be softly ionized. Here, it was confirmed that NaI has no effect as a matrix reagent, because no molecular-related peak of the CD samples in the presence of NaI was observed when silicon-wafers used as the ionization platform. Thus, the PLZT surface would assist the soft laser desorption/ionization of the CD molecules.

Figure 3 shows the SLD mass spectrum of octylphenol polyethoxylate (Triton X-100) observed in the presence of NaI. A series of ion peaks ranging from ca. m/z 400 to 1000 separated by 44 units were clearly observed, with the maximum peak at m/z 625. These ion peaks could be attributed to the sodium adduct ions ($[M + Na]^+$). The peak distribution was comparable to that observed by MALDI-MS.⁸ The number-average molecular weight (M_n) calculated based on the peak distribution was ca. 630, which was in good agreement with the manufacture's information ($M_n =$ ca. 625) and the previous MALDI-MS result ($M_n =$ ca. 620).⁸ Consequently, SLD-MS using the PLZT plate can be used to provide a reasonably accurate molecular weight distribution of Triton X-100.

Although only limited applications have been shown here, we have further demonstrated that other compounds such as synthetic oligomers and several types of additives could also be soft-ionized with almost no fragmentation. At this stage, however, we did not observe higher molecular weight compounds such as proteins and synthetic polymers over m/z 1500. The mass limitation is common problem in the matrix-free SLD-MS. It should be noted that the laser power for the analyte ionization was typically lower than $20 \mu\text{J}/\text{cm}^2$. This setting was almost the same as in

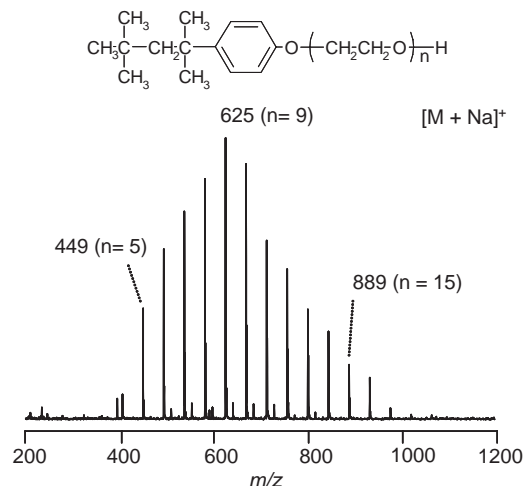


Figure 3. SLD mass spectrum of octylphenol polyethoxylate (Triton X-100) together with the chemical structure.

the case of MALDI-MS measurements using 2,5-dihydroxybenzoic acid (DHB) as a typical matrix used for the measurements of peptides, oligosaccharides, and synthetic polymers. All previous "matrix-free" SLD-MS techniques actually required submicron-structures such as porous silicon,⁵ silicon nanowires,⁶ and nanocrystalline TiO_2 ,⁷ which might essentially act as a matrix for assisting in the desorption/ionization of the analytes. Since the ionization platform used in this SLD-MS study is a flat PLZT plate, another desorption/ionization mechanisms should be considered. Since nanocrystalline TiO_2 could be act as the ionization platform,⁷ TiO_2 framework in the PLZT crystals might effectively assist the ionization. However, nanocrystalline TiO_2 has no pyroelectric nature and it requires mesoporous morphology for the soft ionization. Therefore, we have speculated that pyroelectric phenomenon appearing on the plate surface by UV-laser irradiation might assist the desorption/ionization of the analyte. Although actual ionization mechanisms were not clear at this stage, this finding might provide important knowledge regarding the understanding of soft laser desorption/ionization mechanisms.

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