Quantitative surface-assisted laser desorption/ionization–MS approaches for bioanalysis

“...SALDI-MS using functionalized nanoparticles holds great potential of becoming a primary technique for protein analysis and for MS imaging.”

Keywords: bioanalysis ■ MS image ■ nanomaterials ■ surface-assisted laser desorption/ionization–MS

What is surface-assisted laser desorption/ionization–MS?

MALDI–MS is popular for the analysis of peptides and proteins. However, its quantitation capability is not great due to sweet spots when using organic matrices. In addition, information regarding their complexes cannot be obtained because the analysis is usually conducted under acidic conditions. To overcome these disadvantages, nanomaterials (NMs) have been used to replace organic matrices. When NMs are used in MS, the technique is referred to as surface-assisted laser desorption/ionization–MS (SALDI–MS). This Editorial focuses on the SALDI–MS of biopolymers, including peptides, proteins, DNA and polysaccharides.

Advantages of SALDI–MS

With recent advances in nanotechnology, NMs of various size, shape and composition (most commonly Au, Ag, SiO₂, TiO₂ and Fe₃O₄) have been used for bioanalysis in MS. Organic matrices, such as 2,5-dihydroxybenzoic acid, absorb the energy of the laser light and transfer it efficiently to analytes to induce their desorption and ionization. In addition to playing a similar role to that of organic matrices, bare or functionalized NMs can be used for the concentration of analytes of interest, leading to greater sensitivity and lower MS background. Having large surface areas and high molar adsorption coefficients, efficient desorption/ionization processes are readily achieved. These NMs and their conjugates presenting recognition elements can, in some cases, be used as affinity probes for SALDI–MS to concentrate analytes from complicated samples [1]. For example, TiO₂ nanoparticles (NPs) were used to concentrate catechins from tea brews prior to SALDI–MS using the NPs as a matrix.

SALDI–MS is useful for the analysis of small analytes, because of its low MS background in the low-mass region ($m/z < 1000$). Unlike the organic matrices that form heterogeneous co-crystals with analytes, the NMs can be mixed well with the analytes and form homogeneous sample spots. The decrease in sweet spots during the MS analysis leads to lower spot-to-spot and sample-to-sample variations. As a result, SALDI–MS allows quantitation of biopolymers.

Selection of NMs

The most popular matrices in SALDI–MS are Au and Ag NPs, mainly because preparation is simple. In addition, they interact with thiol-containing analytes through specific Au–S or Ag–S interactions, leading to ease in concentration of the analytes. In other words, Au–NPs can be used as concentration probes and SALDI–MS matrices for bioanalysis. Metallic NMs, metal oxide NMs, semiconductor quantum dots and carbon NMs have been successfully demonstrated for the analysis of proteins. Each type of NM has its advantages and disadvantages over others. For example, Au NMs are favorable for small thiol-containing analytes, while Fe₂O₃ and TiO₂ NMs are useful for phosphate containing analytes. HgTe NMs are suitable for the analysis of small to large analytes (up to 150 kDa), but preparation is difficult and toxicity of the Hg precursor is a concern. Relative to HgTe NMs, CdSe NMs provide better MS resolution for the analysis of proteins, but a lower MS detection range (usually <20 kDa).

SALDI–MS of proteins & peptides

Titania sol-gel films have been employed for the analysis of surfactants, peptides, tryptic digest products and small proteins with molecular

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weights below approximately 24 kDa through SALDI-MS [2]. SALDI-MS using stable and low-thermal conductivity Pt nanoflowers is useful for the analyses of angiotensin I, substance P, insulin and cytochrome C, down to femtomolar levels. SALDI-MS using functionalized CdSe quantum dots with 11-mercaptopundecanoic acid has been applied for the detection of insulin, lysozyme and myoglobin with high resolution, providing a LOQ of insulin of 100 pM with RSD < 10%. Cd analyte adducts are detected, which is believed to be responsible for the increased sensitivity of the analytes. Recently, carbon nanodots prepared from used coffee and tea have been demonstrated as efficient matrices for the analysis of peptides and insulin [3]. Although carbon-based NMs are cost effective, they are not as suitable as HgTe NMs for the analysis of large proteins.

“...SALDI-MS using HgTe nanomaterials has shown great promise for bioanalysis.”

In order to improve the sensitivity, functional magnetic NMs are commonly used for concentration of proteins and peptides, mainly because the concentrated analytes can be easily separated by magnetic separation. Titania-coated magnetic iron oxide NPs ($\text{Fe}_3\text{O}_4@\text{TiO}_2$ NPs) conjugated with target peptides have been used to isolate cytochrome C (10 nM) and myoglobin through employing a magnetic field prior to SALDI-MS analysis. Similarly, sulfonate group-modified FePtCu NMs are useful for the detection of lysozyme in human serum through SALDI-MS.

SALDI-MS of protein complexes

HgTe nanostructures synthesized from Hg and Te salts have a network structure formed from the connection of many nanoscale structures. It is believed that these network structures help the energy transfer between the matrix and analytes. In addition, the low thermal conductivities of HgTe allow generations of high laser-induced temperature even at low laser power, leading to efficient desorption/ionization of proteins. HgTe NMs have been used for SALDI-MS-based analyses of proteins and their complexes with small analytes [4]. This approach provided a concentration LOD of 200 pM for angiotensin I when using HgTe NMs as concentration probes and matrices.

SALDI-MS analysis using HgTe NMs can be conducted across a wide pH range (4–9) and

thus, it has been used to detect protein complexes. For example, MS signals at m/z 86,585 are assigned for the $[\text{IgG} + \text{protein G} + 2\text{H}]^{2+}$ adducts of IgG and G-protein complexes [5].

SALDI-MS of carbohydrates & polysaccharides

SALDI-MS using HgTe NMs have been applied to detect carbohydrates and polysaccharides, including sucrose, cyclodextrins, pullulans (6–10 kDa) and dextran (19.5 kDa) [6]. For these analytes, $[\text{M} + \text{Na}]^+$ ions are the dominant species detected in the mass spectra. This SALDI-MS approach allows quantitative determination of the concentrations of the analytes. The MS profiles of PL-6k and PL-10k are symmetric, revealing the absence of degradation during the SALDI-MS analysis process. From the MS data, the polydispersity indices of PL-6k and PL-10k were determined to be 1.03 and 1.05, respectively, which are lower than that provided by gel permeation chromatography analysis.

SALDI-MS for MS imaging

Imaging MS has been applied to trace and visualize the spatial distribution of analytes and their metabolites in cells and tissues using graphite, Ag and magnetic NMs, without conducting labeling of the analytes [7]. Imaging MS using Ag NPs modified with alkylcarboxylates and alkylamines have been employed for the visualization of fatty acids in mouse liver and retinal samples in a negative ion mode. This approach provided an LOD of 50 pmol for palmitic acid, with a spatial resolution of 10 μm . Using 3.7-nm diameter functionalized magnetic NMs, SALDI-MS allowed localization of lipids and peptides at a resolution of 15 μm in mammalian tissues. Although SALDI-MS provides structural information, relative to optical approaches, its poor spatial resolution still remains challenge.

Conclusion & future perspective

In the authors' view, the selected chemical and biopolymer applications are of particular interest because they clearly show the potential of SALDI-MS for omics, with advantages of sensitivity, reproducibility and simplicity. Because SALDI-MS can be applied in a wide pH range, it can provide wealthy information about complexes such as antibody and antigen, as well as drugs and receptors.

Based on our own experience, we suggest that Au NMs are suitable for the analysis of small analytes (m/z < 1000 Da). Using

internal standards (thiol-modified Au NPs), the SALDI-MS approach can provide excellent reproducibility, allowing quantitative determination of the concentrations of small analytes of interest. HgTe NMs can be used for the analysis of large biopolymers. We have recently shown that SALDI-MS using HgTe NMs is sensitive for the detection of single- and double-stranded oligodeoxynucleotides. This result suggests that SALDI-MS using HgTe NMs may be useful for the analysis of protein–DNA complexes.

Having advantages of quantitation, capability of detection of protein complexes, accurate determination of average molecular weights of polysaccharides, sensitivity and ease in sample preparation, SALDI-MS using HgTe NMs has shown great promise for bioanalysis. In order to further achieve greater sensitivity and lower MS background, concentration of analytes prior to SALDI-MS analysis is suggested. Unlike TiO₂ NMs for phosphate-containing peptides and

proteins, HgTe NMs do not provide specificity for certain analytes. Thus, conjugation of HgTe NMs with aptamers or antibodies may be useful for improved sensitivity for the analysis of biopolymers. With its capability for selective concentration of proteins from complicated samples, SALDI-MS using functionalized NPs holds great potential of becoming a primary technique for protein analysis and for MS imaging.

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