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Nanomaterial-based miniaturized extraction and preconcentration techniques coupled to matrix-assisted laser desorption/ionization mass spectrometry for assaying biomolecules

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ABSTRACT

Within the past decade, intensive efforts have been devoted to the development of core nanomaterials (NMs) with tunable surface properties and their integration into miniaturized solvent-extraction and sample-preparation methods for extraction, enrichment and preconcentration of ultra-trace biomolecules prior to analysis by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). We summarize the most recent bioanalytical developments that employed NMs as effective probes using solvent microextraction in preconcentration methods coupled with MALDI-MS for assaying biomolecules from complex biological samples. We classify the nanomaterial (NM)-based miniaturized extraction/preconcentration methods into four parts: (1) nanoparticle (NP)-based single-drop microextraction; (2) NP-assisted liquid-liquid microextraction; (3) NMs as affinity and concentrating probes; and, (4) NMs as probes for bacterial analysis. We particularly emphasize efforts on NM-based miniaturized extraction and sample-preparation methods coupled with MALDI-MS for bioassays. Finally, we introduce practical applications of the above approaches.

Contents

1. Introduction

Biomolecules, such as peptides and proteins, serve many functions and play key roles in many biological processes and pathways. Proteomic studies have provided experimental and fundamental approaches to explain the information contained in genomic sequences in terms of structure, function, and control of biological processes

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and pathways. In order to probe the new insights to understand protein functions in biological systems, novel mass spectrometry (MS) ionization techniques, such as electrospray ionization (ESI) [1], matrix-assisted laser desorption/ionization (MALDI) [2,3], desorption ESI [4], electrospray-assisted LDI [5], surface-assisted LDI (SALDI) [6], liquid nitrogen-assisted spray ionization [7] and desorption/ionization on porous silicon [8], have been developed for easy identification of various biomolecules, including peptides, proteins and drug metabolites in biological samples. One of the most important studies in proteomics is shotgun proteomics for investigating proteomic differences in cells or tissues [9–11]. The integration between MS and proteomics has made proteomics possible and widely broadened the application of MS-based techniques, leading to a continual development of instrumentation to meet the major challenges in proteomics. However, challenges still exist for qualitative and quantitative analysis of peptides and proteins with a high degree of biocomplexity due to low-abundance (marker) protein signals typically being suppressed by high-abundance background proteins in complex biological samples. Sample-pretreatment techniques have therefore been recognized as a key bottleneck in the bioanalytical process to improve the signal intensities of low-abundance (marker) proteins in MALDI-MS [12,13]. At the same time, proteomics demands efficient MS methods for analysis of ultra-trace peptides and proteins with low limits of detection (LODs) in complex matrices, which can eliminate interferences from the unwanted species with minimal volumes of sample. As biological samples usually derive from cells, protein mixtures or complicated matrices, there is a need to purify the samples from the cells during the sample-preparation stage. Thus, nanomaterial (NM)-based microextraction methods coupled with MS techniques are effectively miniaturized analytical tools for high-throughput bioassays.

Recently, tremendous developments in bioanalytical chemistry *via* the integration of NMs with MS have improved the analysis of a wide variety of biomolecules [14–18]. As a result, new NMs have been used as matrices, extracting or preconcentrating probes for biomolecular analysis in MALDI-MS. Due to their unique optical and physico-chemical properties, NMs are promising analytical probes in miniaturized solvent extractions for well-defined biomolecular assays by MALDI-MS. With the novel uses of NMs in microextraction methods coupled with MALDI-MS for biomolecular analysis, we discuss NM-assisted approaches to sample pretreatment [i.e., single-drop microextraction (SDME), liquid-liquid microextraction (LLME) and affinity probes] to extract or to enrich trace-target analytes prior to MALDI-MS analysis.

2. Properties and analytical applications of nanomaterials

The combination of nanoscience with analytical chemistry for the development of ultra-sensitive detection methods in bioanalytical chemistry is increasingly important in proteomics [15–18]. NMs with typical particle diameters of 1–100 nm are attracting growing interest from the bioanalytical community for their use as sensors [19–22] or analytical probes [23–25] in inorganic, organic and biomolecular assays. NMs exhibit attractive unique physico-chemical and optical properties, which make them ideal candidates for trapping trace-level targets and enhancing their signals in various analytical instruments.

The diversity of NMs is wide and their dimensions can vary over two orders of magnitude (1–100 nm). It is well known that NMs have shown impressive changes in physico-chemical properties [i.e., optical, electrical, thermal, tunable particle size (1–100 nm), large surface area (700–1000 $m^2 g^{-1}$), magnetic, and catalysis] due to their ultra-small size and large surface-area-to-volume ratios. NMs feature unique physico-chemical properties that can be very useful in creating new probes for extraction and preconcentration of ultra-trace analytes, which can improve detection sensitivity with miniaturized sample preparations. Considering these unique properties of NMs, NM-based sample-separation and preconcentration techniques play important roles in many analytical procedures (e.g., to increase analyte concentration and to remove interfering species) prior to target-analyte identification by using UV-visible, fluorescence and MALDI-MS techniques [15–24].

However, all previous reviews have focused on the use of NMs as matrices for the analysis of organic and biomolecules by MALDI-MS, but there has been no review focused on the NM-assisted microextraction techniques coupled with MALDI-MS for bioassays. With this in mind, the main motivation of this review is to offer a rational vision of the main achievements made in NM-based liquid microextraction techniques coupled with MALDI-MS for bioassays.

2.1. Why organic matrices are needed

A wide variety of organic and inorganic materials have been used as matrices in MALDI-MS for analysis of peptides and proteins, since its inception in 1988 [2,3]. With the increasing popularity of MALDI-MS for biomolecular assays, publications increased on using organic derivatives and inorganic NMs as matrices. Two reviews highlighted here include one by Rotello and Vachet, who described the role of the surface chemistry of nanoparticles (NPs) in improving MALDI-MS analysis for cell-membrane lipids, proteins and nucleic acids [18], and others by Chang et al. and He et al., who illustrated how the nature, the size and the concentration of the NPs can help to ionize target analytes by absorbing laser energy, and reducing background signal noise in the low mass region [15,16]. Furthermore, a few reviews, including those by Zenobi and Knochenmuss [26], Marvin et al. [27] and Zou et al. [28], described the use of UV-absorbing organic derivatives as matrices, their technological aspects and working principles in determining the structures of various molecules by MALDI-MS. Recently, Lu et al. reviewed the role of MALDI targets modified with NPs along with organic matrices for concentrating and enriching low-abundance peptides and proteins prior to MALDI-MS analysis [29].

Generally, organic or inorganic matrices can strongly absorb UV laser (N_2) energy at 337 nm, which facilitates proton transfer (organic matrices) or laser-energy transfer (inorganic) for efficient ionization of analytes. Organic and inorganic matrices have drawbacks for the analysis of target biomolecules by MALDI-MS that are well discussed in the above reviews.

Taking into account our previous study [30], and the above reviews, the combination of nanoparticle-conjugated target analytes with the organic matrix system provides a good technical platform to improve laser-absorbing ability, specificity, reproducibility (spot-to-spot and sample-to-sample), and sensitivity for ultra-trace bioassays by MALDI-MS. The desorption-ionization process is complex, involving optical and physico-chemical processes. During it, NMs play many roles as antenna (receiving UV energy from the laser source), preconcentrator and reservoir of energy. The addition of conventional matrices (organic matrices) into NM-conjugated analytes plays a bridging role that relays the energy and functions as proton donor, leading to more efficient UV-energy-transfer for desorption/ionization of target analytes with clean, highly-protonated-ion-dominant mass spectra. Addition of organic matrices into NM-conjugated analytes would be a rational choice to serve as a "reservoir" to lock the energy in the surrounding area of UV-activated NMs, and to act as an efficient means of UV-energy transfer to improve the performance of NP-assisted LPME coupled with MALDI-MS for the production of protonated analyte ions and to reduce the fragments of analytes.