http://dx.doi.org/10.5012/bkcs.2012.33.9.3076

Notes

Selective Analysis of Thiol-Containing Molecules Using Nanoengineered Micro Gold Shells and LDI-TOF MS

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Received May 4, 2012, Accepted June 11, 2012

Key Words : Gold shells, MALDI-TOF MS, Matrix-free, Thiols

Thiol-containing (bio)molecules, such as cysteine (Cys), homocysteine (Hcy), and glutathione (GSH), are recognized to be physiologically important and known to be involved in many diseases, such as leucocyte loss, psoriasis, liver damage, and cancer, depending on changes in the cellular level.[1] Particularly, GSH, which exists in high abundance in biological systems, plays key roles in a variety of biological processes including maintenance of intracellular redox activities and cellular homeostasis, xenobiotic metabolism, intracellular signal transduction, and protecting against oxidative stress and toxic species.[2-4] Accordingly, the detection of thiol-containing molecules in samples requires critical selectivity and high sensitivity. For this purpose, various detection techniques, such as gas chromatography,[5] high performance liquid chromatography,[6] capillary electrophoresis,[7] electrochemical detections,[8] and colorimetric detection with fluorescence[9] or Ellman's reagent (5,5'-dithiobis(2-nitrobenzoic acid) or DTNB), have been developed. However, these biothiols have similar physical properties in terms of polarity and solubility, and almost same chemical reactivity in thiol functionality for tagging optical probes. Thus, these similar physical and chemical properties of biothiols lead to poor selectivity in various detection methods. In this regard, mass spectrometry (MS) has recently been utilized for the detection of thiol-containing molecules, since MS analysis provides molecular weights of analytes which are the intrinsic property of molecules, and therefore, affords direct informations of analytes.[10] In addition, MS analysis can easily discriminate between true analyte and background, and can monitor multiple analytes simultaneously, resulting in reduced false positive signals and multiplexing capability.

Matrix-assisted laser desorption/ionization (MALDI) is a laser-based soft ionization technique used in mass spectrometry. It is widely used for the analysis of biomolecules and macromolecules such as proteins, DNA, organic polymers, and dendrimers. However, analysis of small molecules is hampered by an organic matrix requirement due to interference of a matrix in the low mass region. To overcome this issue, surface-assisted laser desorption/ionization mass spectrometry (SALDI-MS) has been actively studied using various nanoparticles (NPs) and nanostructured surfaces with organic matrix-free format.[11-13] In this paper, we describe a simple method for the selective detection of thiol-containing molecules in mixed samples using organic matrix free laser desorption/ionization-time of flight (LDI-TOF) MS with nanostructure embedded gold micro shells (μAuSs).[14]

μAuSs possess embossed nanostructures on the surface which allows the desorption and ionization of analytes.[15-17] Furthermore, thiol-containing molecules can be selectively captured on μAuSs by way of a specific gold-thiol interaction leading to the successful isolation of thiol-containing molecules in mixed samples. Figure 1 shows the schematic presentation of our strategy for selective extraction and analysis of thiol-containing molecules in mixtures. μAuSs are incubated with a mixed sample and thiol-containing molecules in the sample are selectively adsorbed to μAuSs by the gold-thiol interaction. All other molecules except thiols are then washed away, and the isolated thiol-containing molecules are analyzed directly by LDI-TOF MS without use of organic matrices which affords peaks corresponding

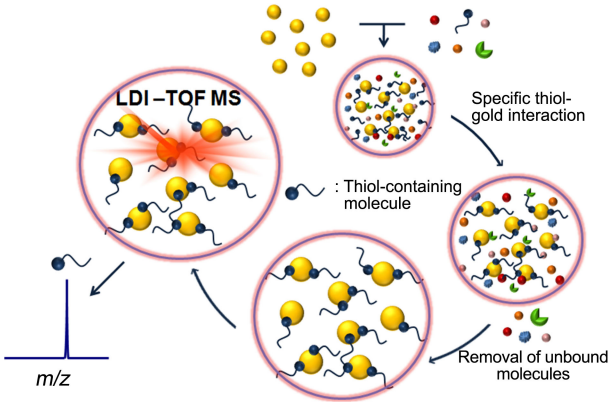

Figure 1. Schematic presentation of the strategy for selective extraction and analysis of thiol-containing molecules in mixtures. Thiol-containing molecules in mixtures are selectively adsorbed to μAuSs by the Au-thiol interaction and all other molecules except thiols are washed away. The isolated thiol-containing molecules are analyzed directly by LDI-TOF MS which affords peaks corresponding to thiol-containing molecules selectively.

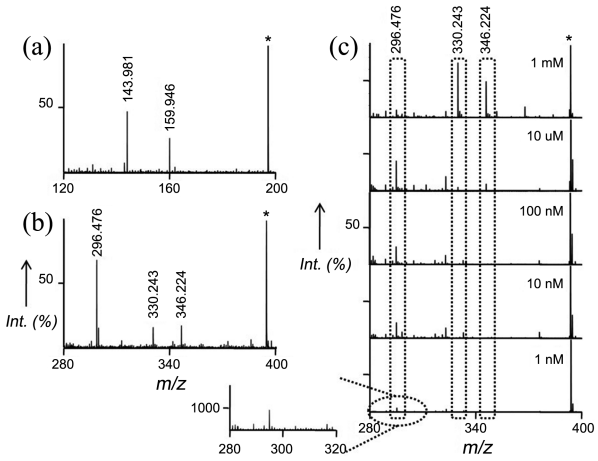

Figure 2. LDI-TOF mass spectra of thiol-containing molecules Cys (a) and GSH (b) using μAuSs. The LDI analysis of GSH could be detected up to 1 nM (40 μL, 40 fmol at S/N = 22) by μAuSs (c). *: gold.

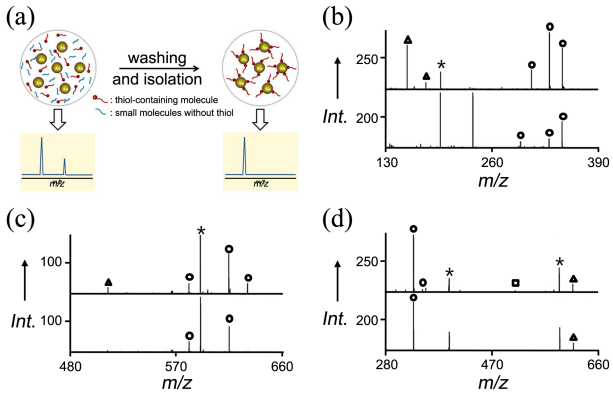

Figure 3. Selective extraction and analysis of thiol-containing molecules in mixtures. (a) Thiol-containing molecules in mixtures are selectively adsorbed to μAuSs and isolated. MS analysis gives the peak corresponding to thiol-containing molecules selectively. (b~d) LDI-TOF mass spectra of a mixture with μAuSs before isolation (top) and after isolation (bottom). (b) histidine (△) and glutathione (○), (c) GRGDS (△) and CGRGDS (○), and (d) glutathione (○), GRGDS (□), and CGRGDS (△). *: gold.

to thiol-containing molecules selectively.

First, we verified the ability of μAuSs for capturing thiols and direct SALDI analysis without organic matrices. 1 mg of μAuSs was suspended in 200 μL ethanol (the number of μAuSs was *ca.* 6×10^5) and 10 μL of the suspension was centrifuged to remove ethanol. 10 μL of analytes, Cys and GSH (1 mM in water), were then added to the μAuSs and incubated for 2 h. The μAuSs were washed with ethanol three times by centrifugation and 1 μL of samples were placed on a target plate and analyzed by MS (each spots on the target plate contained *ca.* 3000 of μAuSs). All measurements were performed using a same laser ablation with an average of 200 shots. As shown in Figure 2(a) and 2(b), mass analyses for Cys and GSH afforded the analyte peaks without significant background interferences and all peaks were clearly assignable; for example, analysis of GSH gave four peaks: $[M+Na\text{-}SH_2]^+$ (*m/z* 296.476), $[M+Na]^+$ (*m/z* 330.243), $[M+K]^+$ (*m/z* 346.224) and $[Au_2]^+$ (*m/z* 393.932). This result indicates that our μAuSs can capture thiols from the solution to the gold surface and the captured thiols can be analyzed successfully by LDI-TOF MS with matrix-free format. For detailed peak assignments of the spectra in Figure 2, see Table S1 in Supporting Information. Next, we examined the sensitivity of μAuSs by measuring the limit of detection (LOD) of GSH as an analyte. We prepared 40 μL of GSH at various concentrations ranging from 1 nM to 1 mM, which were mixed with μAuSs. After 2 h, remaining GSH solution was washed out using centrifugation. The μAuSs were resuspended with 10 μL of water, and then, 1 μL of the suspension was analyzed by LDI MS under the same conditions. LDI mass spectra showed that GSH could be detected up to 1 nM with a S/N ratio of 22 by μAuSs (Figure 2(c)). This result clearly indicates that the μAuSs show excellent detectibility for thiol molecule analysis.

For further extended use of μAuSs for thiol analysis, we tested the selective isolation and detection of thiol-containing molecules from mixtures by harnessing high affinity between gold surface and thiols (Figure 3(a)). For a feasibility study on the selective analysis of thiol-containing molecules, we examined a mixture of histidine and glutathione, a mixture of GRGDS and CGRGDS, and a mixture of glutathione, GRGDS and CGRGDS (equal volume mixture, initial concentration of 10 μM). Before isolation, each mixture was analyzed by μAuSs, which gave mass peaks corresponding to all analytes in the mixture (top, Figure 3(b), (c), (d)). The mixture was incubated with μAuSs for 2 h, after which the suspension was washed with ethanol by centrifugation. μAuSs were resuspended in ethanol, and 1 μL of the suspension was analyzed by LDI MS. Mass analysis after the isolation step gave mass peaks corresponding to the thiol-containing molecules exclusively (bottom, Figure 3(b), (c), (d)). This result suggests that our μAuSs can be used as a selective capture probe and for LDI MS analysis of thiol-containing molecules in complex samples. For detailed peak assignments for the spectra in Figure 3, see Table S2 in Supporting Information.

In this paper, we reported on a simple method for isolation of thiol-containing molecules in the mixed samples using nanostructured gold shells which would facilitate the selective analysis of thiols. In general, both quantitative and qualitative informations are required in the detection of thiol-containing molecules. Our strategy for selective isolation and analysis of thiol-containing molecules using μAuSs and LDI-TOF MS is well suited for this purpose. Our present method utilizes MS which provides the molecular weights of analytes, and therefore, avoids extra steps to determine the identity of analytes. Currently, we are performing an extended study regarding the quantitation of thiol-containing analytes in complex samples such as serum and liver lysate by combining μAuSs and an internal standard. In addition, the use of μAuSs is advantageous over other SALDI materials for analysis of small molecules in terms of high molar absorptivity, large surface area available for analyte loading, and excellent concentration effects.

Therefore, μAuSs provide enhanced efficiency of the absorbance of laser light per unit area and greater possibility of molecular desorption and ionization.

Experimental Section

Materials. μAuSs were obtained from Nomadien Co. (Seoul, Korea, www.nomadien.com). Cysteine was purchased from Deajung Chemicals & Metals Co. Ltd. (Gyonggi-do, Korea). Glutathione was purchased from Sigma-Aldrich (St. Louis, MO). GRGDS, and CGRGDS were custom-synthesized using Fmoc-protected amino acids (AnaSpec, Inc., San Jose, CA, USA).

Analysis of Thiol-Containing Molecules. μAuSs (1 mg) were washed with absolute ethanol by centrifugation (5000 × g, 1 min) and suspended in 200 μL of absolute ethanol. 20 μL of suspended μAuSs were centrifuged to remove the ethanol, and then mixed with 20 μL of analytes (10 μM in ethanol). After incubation for 2 h, this suspension was washed with ethanol three times by centrifugation (5000 × g, 1 min). μAuSs were resuspended in 40 μL of absolute ethanol, and 1 μL of the suspension was pipetted onto a stainless steel 384-well target plate (Bruker Daltonics), dried in air at room temperature, and analyzed directly by MS. Mass analysis was performed using an Autoflex III MALDI-TOF mass spectrometer (Bruker Daltonics) equipped with a smartbeam laser as an ionization source. All of the spectra were acquired with 19 kV accelerating voltage, a 50 Hz repetition rate, and in positive mode with an average of 200 shots.

Acknowledgments. This paper was supported by Konkuk University in 2012.

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