NANO
LETTERS

2007
Vol. 7, No. 10
3023−3025

Tailoring Nanoparticle Surface Chemistry to Enhance Laser Desorption Ionization of Peptides and Proteins

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Received June 20, 2007; Revised Manuscript Received August 18, 2007

ABSTRACT

Gold nanoparticles capped with 4-aminothiophenol have been employed for laser desorption ionization mass spectrometry of biomolecules. We demonstrate that the capped nanoparticles increase ion yields, decrease ion fragmentation, and increase the useful analyte mass range when compared to other nanoparticle systems. These results will allow for further development of nanoparticles with both targeting and enhanced ionization abilities to aid in biomarker screening.

Gold nanoparticles (AuNPs) offer a unique platform for use in laser desorption/ionization mass spectrometry (LDI-MS). For example, size-selected (2, 5, and 10 nm) AuNPs aid in the LDI of peptides having MWs of 500−2500 Da,[1] and AuNPs can be functionalized to capture (pull-down) desired analytes from solution.[2,3] Specifically, Vachet employed monolayer-coated AuNPs to selectively extract peptides from solution based on their isoelectric point, and the peptide/AuNP extract was then mixed with an organic matrix to increase the sensitivity in matrix assisted laser desorption/ionization (MALDI) for a variety of peptide classes.[3] Monolayers on thin films and solid substrates have also been used to aid in MALDI. For example, self-assembled monolayers (SAMs) on gold substrates have been used in MALDI-MS as affinity-capture surfaces,[3−5] for on-probe sample cleanup,[6] and as matrix monolayers.[7] Herein we present the first use of SAM-capped AuNPs for enhanced LDI-MS of biomolecules. The nanoparticles act as chromophores for the laser irradiation, while the SAM promotes production of analyte $[M + H]^+$ ions.

Nanomaterials, such as AuNPs, offer unique advantages for producing analyte ions without using organic matrices.[1,5,8−14] For example, organic matrices produce matrix cluster ions that complicate data analysis for small-molecule analytes. Also, using nanomaterials instead of organic matrices in LDI-MS can reduce sample preparation to simply spotting a small amount of analyte on top of a nanomaterial-presenting surface.[9,10,12]

Owing to the large amounts of sodium citrate needed to stabilize the AuNPs in solution, the positive ion LDI mass spectra obtained using citrate-capped AuNPs contain abundant alkali adducted analyte ions (e.g., $[M + Na]^+$, $[M + K]^+$, and multiple salt adducts).[1] We also find that the ionization efficiency and the energy transfer (evident by the abundance of analyte fragment ions) varies significantly from analyte to analyte. These two factors result in spectral congestion, low $[M + H]^+$ ion production, and poor ion yields for high mass analytes. Previous studies show that a proton donor is key for desorption/ionization of polar biomolecules, e.g., trace levels of glycerol when using metal colloids for LDI[15] and organic matrices for MALDI.[16] *We postulated that capping AuNPs with an efficient proton donor (4-aminothiophenol (4-ATP), as illustrated in Figure 1) would increase the ionization efficiency while maintaining the advantages nanomaterials provide (outlined above).* To test this hypothesis, we employed nanoparticles consisting of a AuNP core capped with an organic SAM for LDI (see Figure 1). Commercially available 5 nm AuNPs (Ted Pella Inc., Redding, CA) were concentrated by 3-fold by using a 10 000 MW cutoff centrifugation filter. Monolayer-capped nanoparticles were prepared by incubating 100 μL of the concentrated AuNPs with 1 mL of 1 mM 4-ATP in a 0.1% trifluoroacetic acid (TFA) solution for 4 h. The particles were then purified by 5 cycles of centrifugation, removal of the supernatant, and resuspension of the particles in 0.1% TFA. Resuspension of the particles was aided by mild sonication. The final resuspension of the particles brought the nanoparticle concentration to 80 nM. Analyte and NPs were mixed at a 500:1 molar ratio, and a 2 μL aliquot was spotted directly onto a stainless steel sample plate, dried under vacuum, and introduced into the mass spectrometer.[17] LDI was performed by irradiating the sample with 1.0−2.5 mJ/cm^2 of 337 nm laser light. For comparison purposes, the following solutions were mixed with analytes as controls: (i) a 100 μL aliquot of concentrated AuNPs diluted to 80 nM AuNP in 0.1%

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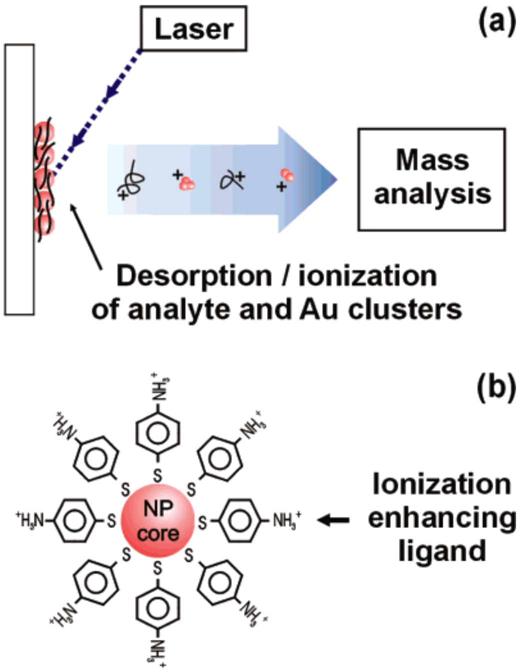

Figure 1. (a) Schematic representation of the nanoparticle based LDI process. Ions are produced by 337 nm irradiation of a sample spot containing a mixture of nanoparticles and analyte. (b) Illustration of a nanoparticle capped with the ionization enhancing ligand 4-aminothiophenol.

TFA, (ii) a 1 mM 4-ATP in 0.1% TFA, and (iii) a 0.1% TFA. For the analytes used in this study, both the 4-ATP and 0.1% TFA solutions produced little or no discernible analyte ion signal above the background.

After investigating a wide range of peptides and small proteins by LDI-MS, it is evident that there is a drastic increase in the amount of $[M + H]^+$ ions produced when using 4-ATP-capped AuNPs compared to citrate-capped AuNPs. In fact, for the peptide ACTH(18−39) (M_r = 2465.20 Da) the increase in $[M + H]^+$ ion signal is greater than 3 orders of magnitude. Figure 2 contains the LDI mass spectra of ACTH(18−39) using both 4-ATP-(on-axis spectrum) and citrate-capped nanoparticles (offset spectrum).

We attribute the drastic enhancement in the $[M + H]^+$ ion signal to two main sources: (i) under the acidic conditions employed (pH = 2), the 4-ATP monolayer is protonated (surface pKa ∼ 6.9),[18] allowing it to act as a source of protons; (ii) the desorption/ionization process using 4-ATP-capped AuNPs produces analyte ions with lower internal energies (i.e., little to no fragmentation). There is also a decrease in the relative amount of gold cluster ions observed between the two spectra (Figure 2). The concurrent increase in $[M + H]^+$ ion signal and decrease in gold cluster ion signal correlates to a more efficient desorption ionization process because the energy may be more effectively channeled into analyte ion production rather then gold cluster ejection. We postulate that the presence of 4-ATP at the surface of the nanoparticle reduces analyte fragmentation by decoupling the analyte from the nanoparticle itself. The presence of the monolayer inhibits direct adsorption of analyte to the nanoparticle surface (i.e., analyte molecules can displace citrate at the nanoparticle surface but not 4-ATP molecules). This lowers the energy required for analyte desorption allowing for a softer desorption/ionization process, in turn producing lower internal energy ions. Similar effects are observed in MALDI when using a binary matrix, i.e., addition of fructose to an organic matrix.[19,20] This decoupling of analyte from the surface may also reduce fragmentation by inhibiting electron transfer from the nanoparticle to the analyte during the LDI process.[21]

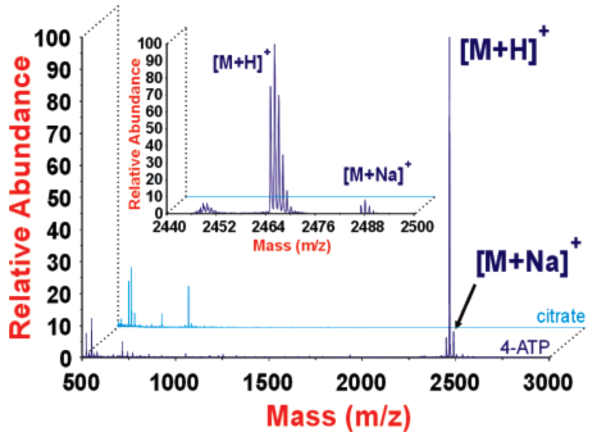

Figure 2. LDI-TOF mass spectra of the peptide ACTH (18−39) (RPVKVYPNGAEDESAEAFPLEF, $[M + H]^+$ = 2465.20 Da) acquired by using 4-ATP-capped AuNPs (dark blue) and citrate-capped AuNPs (light blue).

For most analytes, we also observe a large increase in the ratio of $[M + H]^+$ ions to alkali adducted analyte ions. This increase is a direct result of capping the AuNP with a SAM. The SAM displaces citrate and sodium ions adsorbed at the nanoparticle solution interface. Also, during the purification process, the majority of alkali ions in solution are removed. The reduction of alkali adduction is useful because it simplifies the mass spectra of complex samples, which is advantageous for database searches routinely carried out in proteomic applications.

Another advantage of replacing the citrate with 4-ATP is a 2.5-fold increase in the useful analyte mass range for LDI-MS using AuNPs. Figure 3 contains the LDI-TOF mass spectrum of cytochrome *c* (*m*/*z* ∼12 400 Da) acquired using 4-ATP-capped AuNPs; note that both the singly and doubly charged ions of this protein are observed. LDI-MS control experiments using citrate-capped AuNPs or 4-ATP molecules mixed with cytochrome *c* produced no discernible signal above the background. *We attribute the increase in useful analyte mass range to both an increase in analyte ion production and an increase in analyte ion survival.*[22] This hypothesis is supported by experiments using insulin. For example, LDI-MS of insulin (*m*/*z* ≈ 6 kDa, data not shown) produces a high degree of fragmentation when using citrate-capped AuNPs, while the majority of the protein ion remains intact when 4-ATP-capped AuNPs are used. Again, the insulin $[M + H]^+$ ion signal is improved by several orders of magnitude when 4-ATP-capped AuNPs are employed, while there is only a slight improvement in signal over the control experiment (no AuNPs) when using citrate-capped AuNPs.

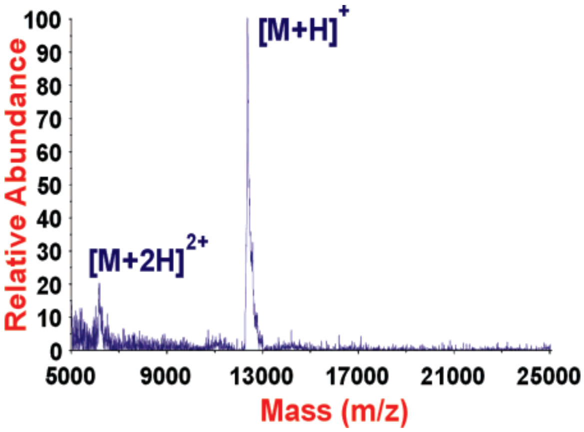

Figure 3. LDI-TOF mass spectrum of cytochrome *c* (cytochrome *c* from equine heart, $[M + H]^+ \sim 12\,400$ Da) acquired using 4-ATP-capped AuNPs from a 0.1% TFA solution.

We have demonstrated that the capping of AuNPs with a SAM of 4-ATP increases ion yield, decreases ion fragmentation, and increases the useful analyte mass range for LDI-MS. Also, the mass spectrum is less complicated due to the drastic reduction of alkali adducted analyte ions. We are currently testing our overall hypothesis that analyte ions produced from 4-ATP-capped AuNPs are lower in internal energy compared to ions produced using citrate-capped AuNPs. For this work, we are exploring fragmentation patterns of analyte ions as a function of laser fluence and SAM composition. Such studies will allow us to develop a model of the AuNPs LDI process.

Currently we are designing monolayer capping agents by systematically altering the locations and chemistries of pendent molecules attached to aromatic thiols. By varying the chemical characteristics of the capping agents employed, we will be able to produce nanoparticles that exhibit an enhancement in both the selectivity of the analyte detection as well the LDI-MS ion yields. For example, we are developing a mixed monolayer-capped particle with both analyte capture and enhanced ionization capabilities. The design criteria for these hybrid particles is (i) enhanced desorption/ionization arising from proton donor or proton acceptor groups in the monolayer and (ii) targeted analyte capture from complex samples.

Targeted hybrid nanoparticles should prove useful in conjunction with our ongoing imaging mass spectrometry experiments.[23] Such a system should have the power to aid in the development of screening assays and early identification of disease biomarkers.

Acknowledgment. This research was supported by a grant from the Robert A. Welch Foundation (A-1176) and the U.S. Department of Energy, Division of Chemical Sciences, BES (DE-FG-04ER-15520).

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NL071469W