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Silver Nanoparticles as Selective Ionization Probes for Analysis of Olefins by Mass Spectrometry

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Laser desorption/ionization (LDI) using silver nanoparticles (AgNPs) is shown to selectively ionize olefinic compounds, e.g., cholesterol, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), and carotenoids. Selective AgNP LDI can be carried out from complex mixtures without the addition of an organic matrix, sample cleanup, or prefractionation. Results presented in this report are the first to demonstrate the selective ionization of specific compounds from a complex mixture using metal nanoparticles.

Recently, there has been growing interest in using nanomaterials to facilitate laser desorption/ionization (LDI) of a broad range of molecules, owing to their unique physicochemical properties.[1−12] LDI using nanoparticles (NPs) differs from matrix assisted laser desorption/ionization (MALDI) in several ways: (i) less complicated mass spectra in the low mass region owing to a decrease in matrix derived chemical noise (matrix ions and adducts),[10] (ii) flexible and relatively simple sample preparation conditions,[2] (iii) a higher tolerance to specific chemical additives, such as those commonly used in biological analysis (e.g., surfactants),[3] (iv) the ability to tailor the chemical properties of NPs using relatively simple derivatization schemes,[9] which can be exploited to (v) modify NPs (metal and silicon based) to selectively capture and ionize analytes on the basis of specific chemical properties (i.e., functional groups).[2,13−17]

Several laboratories have demonstrated that substrate-selective binding strategies can be implemented by treating the modified substrate with a solution containing the analyte of interest, washing the substrate, and then applying an organic matrix prior to analysis by mass spectrometry (MS). For example, Vachet and co-workers utilized mixed monolayer-protected gold nanoclusters to selectively extract and concentrate peptides from dilute solutions.[14] Shen, Brockman, and Teng prepared monolayers on gold nanomaterials that are modified to interact with specific molecules, i.e., oligohistidine-tagged peptides and proteins,[18] hydrophobic peptides,[19] and charged biomolecules;[15] however, all of these methods require the use of traditional MALDI matrixes. On the other hand, Hua and co-workers have utilized ∼160 nm silver nanoparticles to ionize peptides from samples containing large amounts of surfactant,[3] and Lin and co-workers utilized bifunctionalized metal nanoparticles, having both a probe protein and organic matrix component, for solid-phase extraction and ionization of mannose from human plasma samples.[13] These studies illustrate the utility for functionalized nanomaterials to capture and sequester specific molecules and/or remove unwanted salts, chemicals, biomolecules, and/or polymers from samples prior to MS analysis.

The ability to control the size, composition, and electronic properties of nanoparticles provides a means for selective capture and/or ionization of important molecules of a broad range of samples. Previously, we reported that gold nanoparticles can be used to selectively ionize phosphotyrosine over phosphoserine or phosphothreonine containing peptides without the need for an organic matrix or sample cleanup,[2] and we attributed this effect to π−cation interactions between tyrosine and the metal nanoparticle. Here, we exploit the Ag−olefin interaction to selectively ionize specific carotenoids, a sterol, and a lipid from complex mixtures in the presence of AgNPs without additional washing or extraction procedures. Because many classes of molecules contain olefins (e.g., flavanoids, lipids, vitamins, carotenoids, tocopherols, drugs, etc.), the ability to characterize such compounds is of considerable importance. The Ag−olefin interaction has long been used for chemical analysis. For example, Ag−olefin

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interactions enhance Ag cationization by electrospray ionization,[20−23] facilitate olefin transport in supported membranes,[24] as well as separate and quantitate lipids, fatty acids, and triacylglyecerols.[25−28] Moreover, thin silver films have been used to image cholesterol in thin tissue sections of rat kidneys[29] and polymorphonuclear leucocytes.[30,31]

EXPERIMENTAL SECTION

Materials. All peptides (P60, P60 phosphorylated, bradykinin 1-8, [Val^4] angiotensin III, flag, angiotensin II, bradykinin, angiotensin I, substance P, and ACTH (18-39)) were purchased from American Peptide (Sunnyvale, CA) and used without further purification. Cholesterol and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) were acquired from Avanti Polar Lipids (Alabaster, AL). Other chemicals/nanoparticles used in these studies were from the following commercial sources: 20 and 60 nm silver colloids (Ted Pella, Inc., Redding, CA), 2,5-dihydroxybenzoic acid (DHB) (Aldrich Chemicals, Milwaukee, WI), and silver nitrate (Aldrich Chemical, Milwaukee, WI). Although our previous work illustrates different results can be obtained utilizing 2, 5, and 10 nm gold NPs, we do not observe differences in our data when using 20 or 60 nm AgNPs. Freshly squeezed carrot juice was received as a gift from the Jamba Juice Company (College Station, TX).

Sample Preparation. The small unilamellar vesicles employed in these studies were composed of POPC and cholesterol.[32] To prepare these vesicles, the appropriate components (POPC with 5 mol % cholesterol) were first dissolved together in chloroform, dried under a stream of dry nitrogen, and desiccated under vacuum (10^{-3} Torr) for approximately 4 h. After desiccation, the dried lipids and cholesterol were rehydrated in phosphate buffer saline (PBS) solution (pH 7.4) and subjected to 10 freeze/thaw cycles by alternating between rapid immersion in liquid nitrogen followed by immersion in a 30 °C water bath. The small unilamellar vesicle solution was then extruded five times through a polycarbonate filter (Whatman) containing 50 nm pores to produce vesicles of uniform size.

Stock solutions of individual peptides were dissolved in deionized water (18 MΩ, Barnstead, Dubuque, IA) at 1 mg/mL. The peptide mixture solution was prepared by mixing peptides together such that each peptide concentration was between 300−700 pmol/μL. The silver-matrix solution (20 mg/mL DHB in 50:50, v/v, ethanol/120 μM $AgNO_3$ with 0.1% TFA) was mixed 10:1:1 (v/v/v), matrix/peptide mixture/vesicle solution, respectively. The AgNP solution (diluted 4:1, v/v, ethanol/NP solution) was mixed 10:1:1 (v/v/v), NPs/peptide mixture/vesicle solution. For the freshly squeezed carrot juice experiments, the AgNPs were spun down for 20 min at 7000 rpm followed by resuspension in methanol. The AgNPs solution (in MeOH) was mixed 10:1 (v/v), NP solution/carrot juice. A volume of 1 μL of each sample solution was spotted onto the sample plate and dried in vacuo.

LDI and MALDI MS Analysis. All MS experiments were performed on a Voyager DE-STR instrument (Applied Biosystems, Foster City, CA) under optimized conditions in reflected mode utilizing a nitrogen laser at 337 nm. Tandem MS experiments were performed using a 4800 Proteomics Analyzer MALDI TOF/TOF (Applied Biosystems, Foster City, CA). Collision-induced dissociation tandem MS spectra were acquired using air as the collision gas (medium pressure) with a 1 kV collision energy.

RESULTS AND DISCUSSION

The preference for silver binding to olefins is governed by the number, position, and configuration of the double bonds in the target molecule.[25,33] The Dewar model describes the Ag^+−olefin interaction in terms of a σ bond between the free s-orbital of Ag^+ and the olefinic π-orbital and likewise a π-bond is formed by the interaction of the filled d-orbitals of the silver ion and the vacant antibonding π-orbitals of the carbon−carbon double bond. The extent of interaction between overlapping orbitals affects the strength of the Ag^+−olefin interaction; therefore, steric factors have a pronounced effect on the strength of interaction. For example, (i) less substituted double bonds tend to form more stable complexes with Ag^+, (ii) cis conformations will form more stable complexes than trans, (iii) longer hydrocarbon alkene chains are less stable than shorter chains, and (iv) delocalization of electron density, i.e., aromaticity, weakens the interaction.[34]

The data contained in Figure 1 illustrate selective ionization of cholesterol and the lipid, POPC, using 20 nm AgNPs. The sample consists of a mixture of 10 peptides, POPC, and cholesterol (see Table 1), which was analyzed by LDI MS using a mixed matrix composed of DHB and silver nitrate (Figure 1A) and only AgNPs (Figure 1B). The MALDI mass spectrum contains ion signals corresponding to all 12 species present in the mixture. Note that the only cholesterol ion observed in the MALDI spectrum involves a neutral loss of water, specifically [cholesterol $- H_2O + H]^+$ ion (m/z 369.3), and the intact species [cholesterol $+ Ag]^+$ is not observed. Conversely, the mass spectrum of the same 12 component mixture ionized using AgNPs contains ion signals corresponding to silver clusters (Ag_2^+ and Ag_3^+, m/z 213.8 and 320.7, respectively), [cholesterol + Ag]$^+$ (m/z 493.3) and [POPC + Ag]$^+$ (m/z 866.8). Note also that [M + Na]$^+$ ions are not observed in the mass spectrum for AgNPs, even though there is a high salt concentration (~60 mM). LDI spectra of samples that are not treated with AgNP or organic matrix contain no discernible signal above the background. Although Hua et al. reported that LDI using ~160 nm AgNPs facilitates ionization of peptides, we do not observe ion signals for peptides using 20 or 60 nm AgNPs.[3] Quite possibly these differences in peptide

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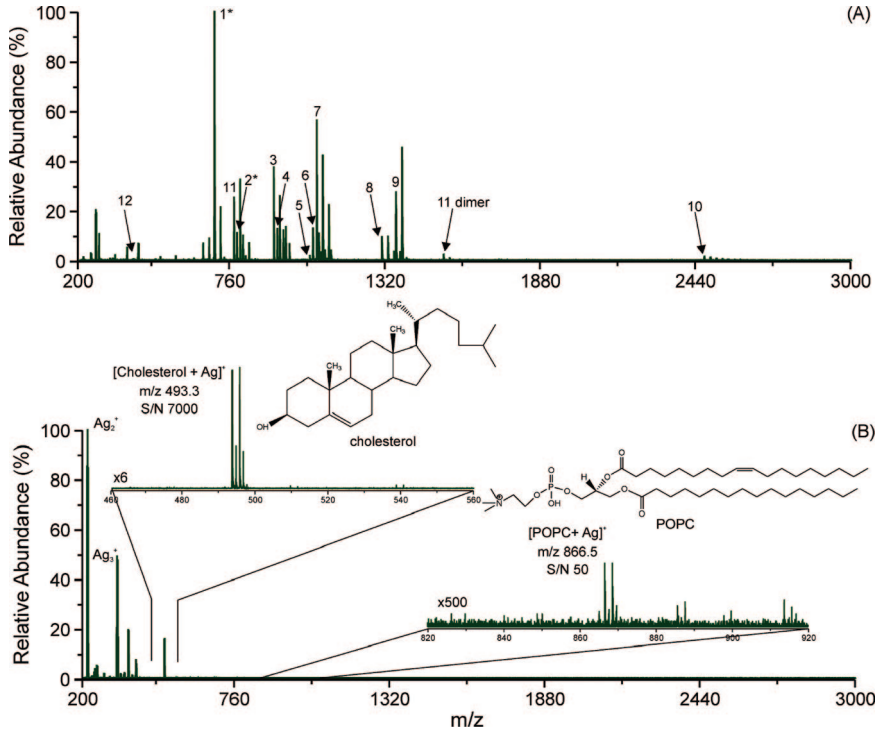

Figure 1. MALDI time-of-flight mass spectra of the 12 component mixture obtained using (A) DHB with 120 μM $AgNO_3$ and (B) 20 nm AgNPs. The $[M + H]^+$ or $[M + Na]^+$ (denoted by a "$*$") ions of each peptide is numbered in the spectrum and identified in Table 1. A high abundance of $[M + Na]^+$ ions (unlabeled peaks) are observed owing to the PBS buffer used to prepare the small unilamellar vesicles. Structures for both cholesterol and POPC are provided.

Table 1. List of Peptides, Lipid, and Sterol in the 12 Component Mixture

	peptide(p)/lipid(l)/sterol(s)	peptide sequence/ chemical composition	matrix obsd m/z	AgNPs obsd m/z	amount spotted on plate (pmol)
1	P60[c] (p)	Ac-IYGEF-NH_2-NH_2[a,b]	690.7		62.3
2	P60 phosphorylated[c] (p)	Ac-IpYGEF-NH_2[a,b]	770.8		55.6
3	bradykinin 1-8 (p)	RPPGFSPF	904.0		46.1
4	[Val^4] angiotensin III (p)	RVYVHPF	917.1		45.4
5	flag (p)	DYKDDDDK	1013.0		41.1
6	angiotensin II (p)	DRVYIHPF	1046.5		39.8
7	bradykinin (p)	RPPGFSPFR	1060.6		39.3
8	angiotensin I (p)	DRVYIHPFHL	1296.7		32.1
9	substance P (p)	RPKPQQFFGLM-NH_2[b]	1347.7		30.9
10	ACTH (18-39) (p)	RPVKYPNGAEDSAEAFPLEF	2465.7		16.9
11	16:0−18:1 phosphocholine (l)	$C_{42}H_{83}NO_8P$	760.6	$[M + Ag]^+$ 866.5	274.2
12	cholesterol (s)	$C_{27}H_{46}O$	$[M - H_2O + H]^+$ 369.4	$[M + Ag]^+$ 493.3	12.9

[a] Ac = acetylated. [b] NH_2 = amidated. [c] Only $[M + Na]^+$ species observed.

ionization efficiencies from AgNP can be attributed to nanoparticle size effects. For example, we previously reported nanoparticle size dependent ion yields for peptides using AuNPs, and we have found that the presence of various salts (especially oxyanions), trace amounts of organics (various alcohols, glycerol, and surfactants), as well as particle size strongly influence LDI ion yields.[2,35] We are currently investigating the effect of competitive interactions on peptide AgNP LDI.[36]

We interpret these results as evidence that the addition of the AgNPs facilitates selective ionization of cholesterol and POPC from the 12 component mixture. In addition to demonstrating the preference for silver to complex with olefinic bonds,[25] the data suggest a preference for forming $[M + Ag]^+$ ions of cholesterol over POPC (as indicated by the high relative abundance and signal-to-noise values of the cholesterol signal compared to that of POPC, see Figure 1). This observation is consistent with the Dewar model. That is, the ring restricted double bond in cholesterol is likely more accessible than is the double bond of POPC, which is surrounded by freely rotating long hydrocarbon chains. Table 1 summarizes the ion signals observed in the spectra contained in Figure 1, and the observed m/z signals of the species detected in the sample mixtures which utilized both the silver doped MALDI matrix and AgNPs.

Selective ionization of olefins using AgNPs is also illustrated by analysis of freshly squeezed carrot juice (Figure 2). Although carrot juice is a complex mixture composed of vitamins, minerals, terpenoids, lipids, carbohydrates, sugars, proteins, carotenoids,

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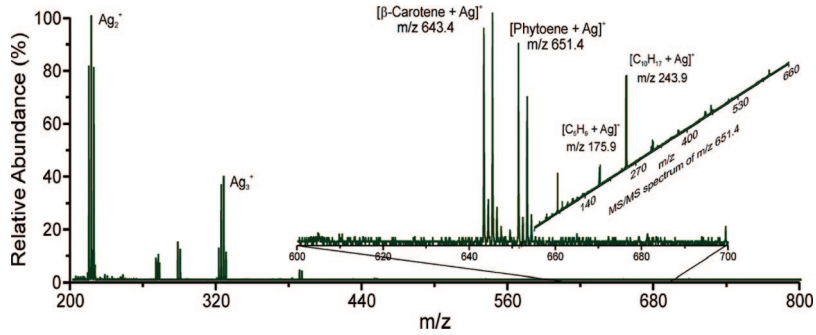

Figure 2. LDI time-of-flight mass spectrum of freshly squeezed carrot juice obtained using 20 nm AgNPs. $[M + Ag]^+$ ions of β-carotene and phytoene are observed in the mass spectrum. The inset contains the tandem MS spectrum for the phytoene ion with the two predominate fragment ions, $[C_5H_9 + Ag]^+$ and $[C_{10}H_{17} + Ag]^+$, labeled in the spectrum.

and amino acids,[37] the AgNP LDI spectrum contains relatively few ion signals, whereas the MALDI mass spectrum is congested (data not shown). In the region of interest, only two signals are observed in the AgNP LDI spectrum, and these signals correspond to a carotenoid and a carotenoid precursor, specifically [β-carotene + Ag]$^+$ and [phytoene + Ag]$^+$, m/z 643.3 and 651.4, respectively. These assignments were validated using collision induced dissociation (MS/MS) of the parent ions. Specifically the characteristic fragment ion [β-carotene − 92 + Ag]$^+$ was observed for β-carotene (data not shown) and two predominant fragment ions, $[C_5H_9 + Ag]^+$ and $[C_{10}H_{17} + Ag]^+$, were observed for the phytoene ion (Figure 2, inset).[38,39] The Ag^+ affinity toward olefinic bonds is further supported by results obtained for pure samples of amphotericin B (m/z 1030.4), folic acid (m/z 548.1), and β-carotene (m/z 643.3) (data not shown). The ion signals observed for these analytes consist exclusively of the $[M + Ag]^+$ ion.

CONCLUSION

We have successfully utilized 20 and 60 nm AgNPs to facilitate the ionization of analytes which contain olefins. To illustrate this point, cholesterol and POPC were selectively ionized from a 12 component mixture, and the data suggest that silver shows preferential binding with cholesterol over POPC. Also, two carotenoids, β-carotene and phytoene, were selectively ionized from a sample of carrot juice using AgNPs. These results demonstrate the ability to use AgNPs to selectively ionize cholesterol, POPC, β-carotene, and phytoene from complex mixtures. The ability to selectively ionize specific chemical species using a sample preparation that introduces minimal chemical noise is especially relevant to biomarker detection or imaging MS, where analysis often requires searching for low-abundance analytes, such as steroid or vitamin analysis, in complex mixtures. Designing other metal or mixed-metal NPs to observe similar selectivity trends are currently being explored. For example, metals (i.e., Cu(II), Ni(II), Zn(II), Co(II), Fe(III), Ga(III), etc.) that are typically used to separate proteins and peptides could be used for selective ionization.[40−42] Tailoring the chemical composition of NPs toward binding specific chemical species provides many avenues to utilize known chemical interactions for compound specific LDI-MS.

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