Gold nanoparticle-enhanced secondary ion mass spectrometry and its bio-applications

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1. Introduction

Enhancing the signals of characteristic molecular or quasi-molecular ions from biomolecules is essential to the analysis of many biological systems because it allows for easier identification of the biomolecules. To this end, the electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) techniques have been regarded as powerful tools for analyzing biomolecules that would otherwise not be easily detected [1]. Recently, as an alternative to these techniques, time-of-flight secondary ion mass spectrometry (ToF-SIMS) has been proven to be an effective method for surface analysis as well as for surface imaging due to its surface sensitivity and chemical specificity without requiring a matrix [2].

Broad biological applications of the ToF-SIMS analysis, however, are hindered by its limited mass range and low mass signals, but to date several approaches have sought to increase the secondary molecular ion yield of biomolecules to improve the mass range. One approach makes use of polyatomic ion analysis [3] and involves the incorporation of various signal enhancers such as a matrix or metal ion [4–7]. However, the difficulty of homogeneously and selectively depositing signal enhancers particularly on a biochip surface is reflected in the poor reproducibility of the resultant signal. To further expand the use of ToF-SIMS in the biotechnological fields, a strategy that includes a method for homogeneously distributing signal enhancers is needed to induce efficient molecular ion emission of biomolecules.

In this report, we present a new methodology for enzyme assay using a sensitive and selective gold nanoparticle (AuNP)-enhanced ToF-SIMS technique on self-assembled monolayers (SAMs). It was reported recently that the use of size-selected (2–10 nm) gold nanoparticles (AuNPs) as a matrix in laser desorption ionization mass spectrometry (LDI-MS) enabled the selective and sensitive detection of phosphopeptides [8]. Yet, until now, no study has been done of the use of AuNPs in detecting biomolecules in ToF-SIMS. In our AuNP-enhanced ToF-SIMS technique, termed NE-SIMS, AuNPs are homogeneously attached onto SAMs to bind targeted analytes...
on various solid substrates and then to act as an enhancer for the generation of secondary ions of analytes. Since this NE-SIMS approach is sensitive to even minor alterations of a small chemical group or moiety in biological molecules, many synthetic peptide substrates for signaling enzymes can be profitably used for SIMS analysis on a chip surface.

2. Secondary ion signal enhancement of peptides on AuNPs

The advantages of the NE-SIMS technique are the homogeneity of the AuNPs and oriented immobilization of the analytes. In this work, AuNPs were synthesized in the presence of citrate by reduction of HAuCl₄ and then easily attached onto amine-ended SAMs via electrostatic interaction. Assuming that deposition of AuNPs is governed by random sequential adsorption, the surface density (3.3–3.5 pmol/cm²) of the 3 nm-AuNPs, which was measured by quartz crystal microbalance (QCM), was found to be the density of the sub-monomolayer. Yet, the surface of the AuNPs provided more binding sites for the peptide substrates than that of SAMs [9]. For orientation, the peptide (Ac-IYAAPKKGGGGC) was immobilized onto the amino groups of the SAM (Fig. 1(a)) or onto the surface of the AuNPs via a cysteine residue at one extremity (Fig. 1(b)). The sulfhydryl groups at the cysteine provide direct attachment to either the terminal group of SAMs or the surface of gold nanoparticle, enabling the vertical orientation of the peptide on surfaces [10,11]. The peptides on the AuNPs were subjected to analysis by ToF-SIMS (ION-TOF GmbH, Germany) with 25-keV Au⁺ (or Bi⁺) primary ions.

In contrast to the low peak intensity of the peptides on SAMs where AuNPs are absent (Fig. 1(a)), peptides immobilized on AuNPs showed an enhanced secondary ion mass signal (Fig. 1(b)). When the cross-linker (SSMCC; sulfosuccinimidyl 4-((N-maleimido)methyl) cyclohexane-1-carboxylate) was used in Fig. 1(a), the mass ion signal of the peptide was critically affected by the relatively strong signals from the cross-linker, resulting in much reduced ion signal. On the other hand, the AuNPs can enhance the peptide signals without requiring a cross-linker. The peptide signal on the AuNPs increases SI yields by factors of 6–7 than the peptide signal on the surface without AuNPs. However, the strength of signal enhancement is generally dependent on the analyzing time, the peptide sequence, the surface area of gold nanoparticle and so on. In addition to oriented immobilization, direct adsorption onto AuNPs of peptides that lack cysteine also showed an enhanced quasi-molecular ion mass signal of [M+H⁺] [9]. The mechanism behind the enhanced secondary ion emission of peptides [M+H⁺] by using AuNPs may be similar to the mechanism observed for peptides on a bare gold surface, i.e., protonation due to a reaction between a neutral peptide and a proton during high-energy events followed by collision cascades. Although it has been reported that cluster primary ion beams have greater SI yield than that of single atomic ion at equal primary ion doses, the mono-atomic beam (Au⁺ or Bi⁺) in the present study allowed for higher SI yields of peptides, compared to the cluster-ion beams (Au₃⁺, Bi₃⁺ or Bi₃²⁺). Interestingly, rather than the [M+H⁺] formation, the oriented peptide with a cysteine residue formed a quasi-molecular ion of [NH–SH–COOH]⁺, which was due to the loss of small functional groups from the analyte molecule [12]. Furthermore, a post-deposition of AuNPs over the peptides led to an inhomogeneous distribution and weak surface binding force of the AuNPs, resulting in no distinct peaks (data not shown). This would explain the absence of a signal, as it suggests that the peptides do not diffuse onto the AuNPs surfaces. This diffusion process was believed to be a necessary condition for signal enhancement by gold deposition on polymer [5] or bio-samples [13].

The AuNP-enhanced ion signal was significantly more dependent on the surface area of the gold, rather than on the surface density of the peptide. For example, despite similar surface densities, the peptides on AuNPs displayed a higher secondary ion emission than those on the SAMs that were unattached to AuNPs, and when the surface area of the gold increased, the intensity of the

![Fig. 1. Gold nanoparticle-enhanced secondary ion mass spectrometry (NE-SIMS). Secondary ion mass signal of peptide was more enhanced on (b) gold nanoparticles (AuNPs) than (a) the self-assembled monolayers (SAMs).](image-url)
peptide ion peak increased exponentially [9]. We previously reported that the increase of secondary ions of peptides was by one order of magnitude than what was seen for other SAMs, such as 11-amino-1-undecanethiol (MUAM), mercaptopropionic acid (MPA), mercaptoundecanoic acid (MUA), and mercaptonicotinic acid (MNA) without AuNPs [9]. Indeed, gold nanoparticles on a SAM can play a pivotal role both as signal enhancers and as linkers that provide binding sites for the peptides. When compared with the analyte/matrix ratio (1:10^3–10^6) in a matrix-enhanced SIMS [4], the ratios of analyte to AuNP were estimated to be 3:1–9:1 by surface plasmon resonance (SPR) and QCM [9], meaning that AuNP is a highly effective signal enhancer. Concurrent with this result, it is noteworthy that AuNPs in LDI-MS were used as an effective matrix [8]. This enhancing property of AuNPs allowed a better chemical imaging of patterned peptides with a rapid scanning rate and sub-micron spatial resolution, which are properties superior to those of the MALDI technique [14]. In this regard, our NE-SIMS method is useful for identifying the location of molecules by imaging the molecular or quasi-molecular ions on a defined area with an unambiguous assignment.

It is likely that AuNPs of different sizes have differing mass limits of peptides. For example, compared to the use of 3 nm-AuNP, the use of 5 nm-AuNPs led to an increase in the detectable mass limit of the peptides and enhanced the secondary ion signal (data not shown). This was probably caused by the delicate balance between the binding density of the peptide and the surface area of the AuNPs. Small AuNPs would seem to be the appropriate choice for NE-SIMS due to their relatively high surface coverage, but binding accessibility and the resultant surface density of analytes also need to be taken into account in the strength of the secondary ion signal emission. Regarding mass imaging in our study, signals of peptides heavier than 1500 Da could not be enhanced, even under optimal conditions. Still, we anticipate that by manipulating the size of the AuNPs for an increased surface area and by controlling the spacing of each AuNP, we can improve the secondary ion yield of peptides for up to a relatively high mass range.

3. Applications using NE-SIMS

AuNP-enhanced peptide signals are useful for assaying the activity of an enzyme. For this purpose, there are many short peptide substrates that can specifically interact with numerous enzymes in vivo and in vitro as stable substrates [15]. As an application of NE-SIMS, we attempted to track phosphorylation by using a known peptide substrate. As proof-of-concept, we first examined SIMS spectra of an acetyl-formed peptide (e.g. Ac-IYAAPKKGGGG as a substrate for Abl kinase) and its synthetic phosphorylated form (e.g. Ac-IYpAAPKKGGGG). The characteristic peaks corresponding to the two peptides were distinctly observed at [MH–SH–COOH]+ and [MH–SH–COOH + HPO3]+ with a difference of 80 Da, due to the presence of the phosphate group (HPO3). Likewise, the kinase reaction generated a phosphorylated signal with a decrease in original peak intensity of the peptide (Fig. 2). In addition to the quasi-molecular mass ion, the peaks of [Tyr–COOH + HPO3]+ and [PO2]−, [PO3]−, and [H2PO4]− were additionally observed after the kinase reaction in the positive and negative modes, respectively [12].

For quantification of the kinase assay, phosphorylation efficiency was determined by ratiometry between the original unphosphorylated peak and the phosphorylated one within a single spectrum. As a result, maximum efficiency by kinase reaction was estimated to be 70%, which was achieved with the optimization of orientation effect and the addition of several linker residues to the peptide. The use of a peptide substrate without cysteine residue (i.e., Ac-IYAAPKKGGGG) or without a spacer (i.e., Ac-IYAAPKKC) at the terminus of the peptide led to a significant decrease of phosphorylation efficiency to the level of 10–30% (data not shown). This result strongly suggests that surface orientation and length of peptide substrate are crucial in the NE-SIMS.
4. Conclusions

The use of gold nanoparticles significantly enhanced the secondary ion emission of peptides in our study of NE-SIMS and its applications. Based on the mass changes of peptide substrates that reacted with enzymes, the NE-SIMS approach enabled the assay of the protein kinase and its inhibition by using mass spectrum or images of microspotted peptide substrates. This approach is a promising tool for the assay of various enzymes as well as for the screening of their inhibitors with high sensitivity in a high-throughput manner.

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