Direct and Real-Time Surface Analysis and Imaging of Biological Samples by Probe Electrospray

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Recently, we have developed the probe electrospray ionization (PESI) that uses the solid needle. In this system, the probe needle moves up and down along the vertical axis by a motor-driven system. At the highest position of the probe needle, electrospray is generated by applying the high voltage. In this study, we used PESI directly to the biological samples such as urine, mouse brain, mouse liver, salmon egg, artificial salmon egg, and fruits (orange, banana, etc.). Strong ion signals for almost all the samples were obtained. The amount of liquid sample picked up by the needle is as small as pL or less, making the PESI the most promising non-invasive technique for detecting biomolecules in a living systems such as cells. Therefore, PESI may be useful as versatile and ready-to-use semi-on line analyses in the fields of surface chemistry, medicine, pharmaceutical, agriculture, food science, etc.

1. Introduction

Electrospray ionization mass spectrometry (ESI-MS) is widely used for the analysis of biological molecules, especially peptides and proteins.[1,2] Furthermore, ESI-MS is now recognized as one of the most sensitive methods, by lowering flow rate to achieve a higher detection limit.[3] For example, Wilm and Mann developed a low-flow sprayer for the analysis of small volumes of peptide solutions at a flow rate of low tens of nL/min.[4,5] According to them, small droplets have several advantages for the analysis. One of them is a high surface-to-volume ratio, which makes a large proportion of analyte molecules available for desorption.

Electrospray generally employs a continuous-flow technique using a capillary. There may be technical limit for capillaries with the diameters smaller than 1 μm because it becomes increasingly difficult to spray the liquid from the smaller size capillary due to the surface tension of the liquid, in addition to the clogging problem. To circumvent these intrinsic problems for electrospray using narrow-bore capillaries, Shiea et al. developed several different electrospray designs that effectively use solid probes.[6-10] By using tungsten oxide wire,[10] they succeeded in treating as little as 50 nL of the methanol solution at its tip. Under the influence of a high electric field, electrospray from a Taylor cone on the adhered methanol solution was observed and ~50 fmol of the peptides were successfully detected.[10]

Recently, we have developed the probe electrospray ionization (PESI) technique that uses the solid needle for the electrospray.[11,12] It was found that the addition of salts or acids to aqueous solution led to dramatic enhancement of the protein ion signals. In this work, PESI was applied directly to various biological samples without any special sample preparations. It was found that PESI gave reasonably strong ion signals for almost all of the biological samples.

2. Experimental

The general experimental procedures are similar to those described in our previous paper.[11] Figure 1 displays the probe electrospray system. The needle was moved up and down the vertical axis using a motor-driven system. The bottom position of the needle was adjusted just to touch the surface of the sample put on a sample stage.

The needle and the sample stage were kept at the ground potential when the needle made a contact with a sample. When the needle was moved up and reaches the highest position, a high voltage of about 3 kV was applied to the needle. The distance between the top and bottom positions of the needle was adjusted to be 5~10 mm. The strongest ion signals were obtained at the top position of the needle tip 3~5 mm apart from and 3 mm above the apex of the ion sampling cone of the mass spectrometer. The ion signal abundances are sensitive to the needle position and some care must be taken for obtaining the most abundant ion signals depending on the experimental conditions.

Disposable acupuncture needles (Seirin, Shizuoka, Japan) with ~350nm tip radius were used as electrospray emitters throughout the PESI-MS experiment.

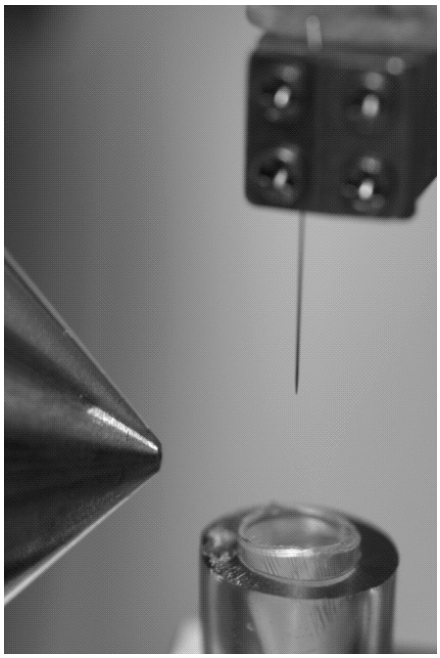

Fig. 1 Probe electrospray system.

The probe for PESI is monitored by a CCD camera (Toshiba, type IK−52V). This was found to be a very powerful technique for the optimization of the probe position and also for the observation of the contaminants remained on the tip of the probe.

The ions formed from the electrosprayed liquid droplets were sampled through the ion sampling orifice with the diameter of 0.4 mm into the vacuum chamber and mass-analyzed by an orthogonal time of flight mass spectrometer (AccuTOF, JEOL, Akishima, Japan). Mass spectra were measured using the ADC/continuous averager ion detection system. The PESI mass spectra are obtained with the ion collection time of 0.5 s with 3 Hz of the probe motion. Thus the PESI mass spectra correspond to those obtained by a single sample loading by the needle probe.

3. Results and Discussion

Direct analysis of biological samples by electrospray is usually regarded as a difficult task. Normally, multi-step sample preparations are necessary to achieve satisfactory performance. Here, we developed PESI that can be applicable to various wet biological samples (e.g., urine, mouse brain, mouse liver, cerebrospinal fluid, aqueous humor serum, human blood, sashimi of fish, apple, plum, pumpkin, orange, banana, strawberry, water melon, radish, etc.) without any special sample preparations.

As the normal human urine contains ~100 mM inorganic salts (e.g., NaCl, KCl, etc.), some sample preparations such as dilution by methanol, dialysis, or ultrafiltration are required for the conventional electrospray. In contrast, PESI is found to be readily applicable to human (and also mouse) urine without any sample preparations. As is well known, electrospray is more sensitive to the more surface-active molecules because they are enriched on the surface of the charged droplets leading to the preferential formation of gaseous ions via the off-spring droplet fission. When the needle is pulled up from the sample solution, the more hydrophobic molecules enriched on the liquid surface must have larger chance to adhere to the needle surface than hydrophilic molecules. In this respect, the sampling process in PESI may be similar to that of Langmuir-Blodgett film formation on the substrate surface.

Despite of the relatively high concentration of salts in urine, little salt cluster ions are observed. Very low intensities of salt cluster ions in PESI mass spectra are found to be the case for almost all of the biological samples measured (e.g., celebrospinal fluid, human blood, aqueous humor**,** serum, mouse brain, mouse liver, etc).

Wilm and Mann studied the analytical properties of the nanoelectrospray ion source.[5] The measured flow rate for used capillaries is 20-40 nL/min and the predicted droplet diameter is less than 200 nm, about 100-1000 times smaller than the volume of the 1-2 μm droplets generated by conventional electrospray source. They measured the electrospray spectrum of a 10^{-6} M peptide (MDMSSKDESVDYVPMLD-NH_2) in an aqueous 10^{-1} M NaCl solution. The solution was electrosprayed without any nebulizer or sheath flow assistance. The peptide was discernible but the spectrum was dominated by the salt cluster ions. Very little salt cluster ions appearing in PESI mass spectra may partly be due to the formation of finer droplets by PESI than by nanoelectrospray. In order to obtain more detailed information on the ion formation mechanism by PESI, observation of the electrospray plume generated by PESI using a pulsed 532 nm YAG laser is made.

Figure 2 shows the mass spectra for mouse brain analyzed by PESI-MS (central panel). In this measurement, the depth of the needle probe stuck into the mouse brain surface was changed step-by-step with 2.5 μm interval. The number *n* displayed in each mass spectrum denotes the depth, i.e., the depth from the surface to the tip of the needle is given by $n \times 2.5\mu m$. In Fig.2, one can see that the mass spectra are highly dependent of the depth of the sample stuck by the needle. For

example, the protonated phosphatidylcholine $[PC34:1 + H]^+$ was observed as a base peak at the surface of the sample. With increase of needle probing depth into the sample, the intensity of the potassiated phosphatidylcholine $[PC34:1 + K]^+$ increases and becomes stronger than that of $[PC34:1 + H]^+$ as shown in the left panel of Fig.2. This clearly shows the ability of PESI for the depth profiling of the biological tissues.

The right panel of Fig.2 shows the images of the damaged parts made on the tissue surface by sticking the sample with n = 2, 5, 7, and 9 (scale bar in the figure: 10μm). The size of the hole with $n = 2$ (i.e., invasion depth ≈ 5μm) is a few μm in diameter but still strong enough ion signals can be obtained as shown in the central panel in Fig.2. This indicates that PESI can be applied to the surface imaging of the biological samples with the space resolution of μm or even smaller.

Since PESI is semi-on line analytical method, it may be applicable to monitor the reaction kinetics taking place in the samples with the time resolution of $1/N$ s with probe frequency of N Hz.

4. Concluding Remarks

The conventional electrospray using capillaries needs long flashing time to prevent the contamination from previous samples. The capillary used for nanoelectrospray with inner caliber of less than 20 μm were too small to use it routinely, as it is easily clogged by minute particulate matter contaminated in the samples. Besides, an unstable electrospray is usually obtained when spraying with water-rich solution. In contrast, PESI allows rapid and sensitive analysis of unprocessed native samples under ambient conditions (water is a natural solvent). PESI can be readily applicable to biological samples containing salt concentrations of ~150 mM without any clogging problem and special sample preparation. The high surface tension of water makes the thickness of the liquid film deposited on the metal surface thinner than ~1 μm.[11,12] A very small amount of the sample seized on the tip of the needle probe (less than ~1 pL)[11,12] can be electrosprayed. It was found that almost all of the biological samples measured gave ion signals strong enough for the single sample loading on the probe tip except for the high viscous biological samples.

The sampling procedure in PESI can be envisaged as the Langmuir-Blodgett membrane formation on the substrate surface. In this respect, PESI can be regarded as a surface-sensitive analytical method for liquid-like samples. PESI is a semi-on line technique and it can be applicable to monitor the reaction kinetics taking place in solution. PESI has an ability to collect ultratrace quantities of samples for adequate ion signals. Due to its high sensitivity, PESI may be applicable to the nano-scale imaging of the biological tissues and even to the cells. Further investigation on this respect is in progress in our laboratory.

5. References

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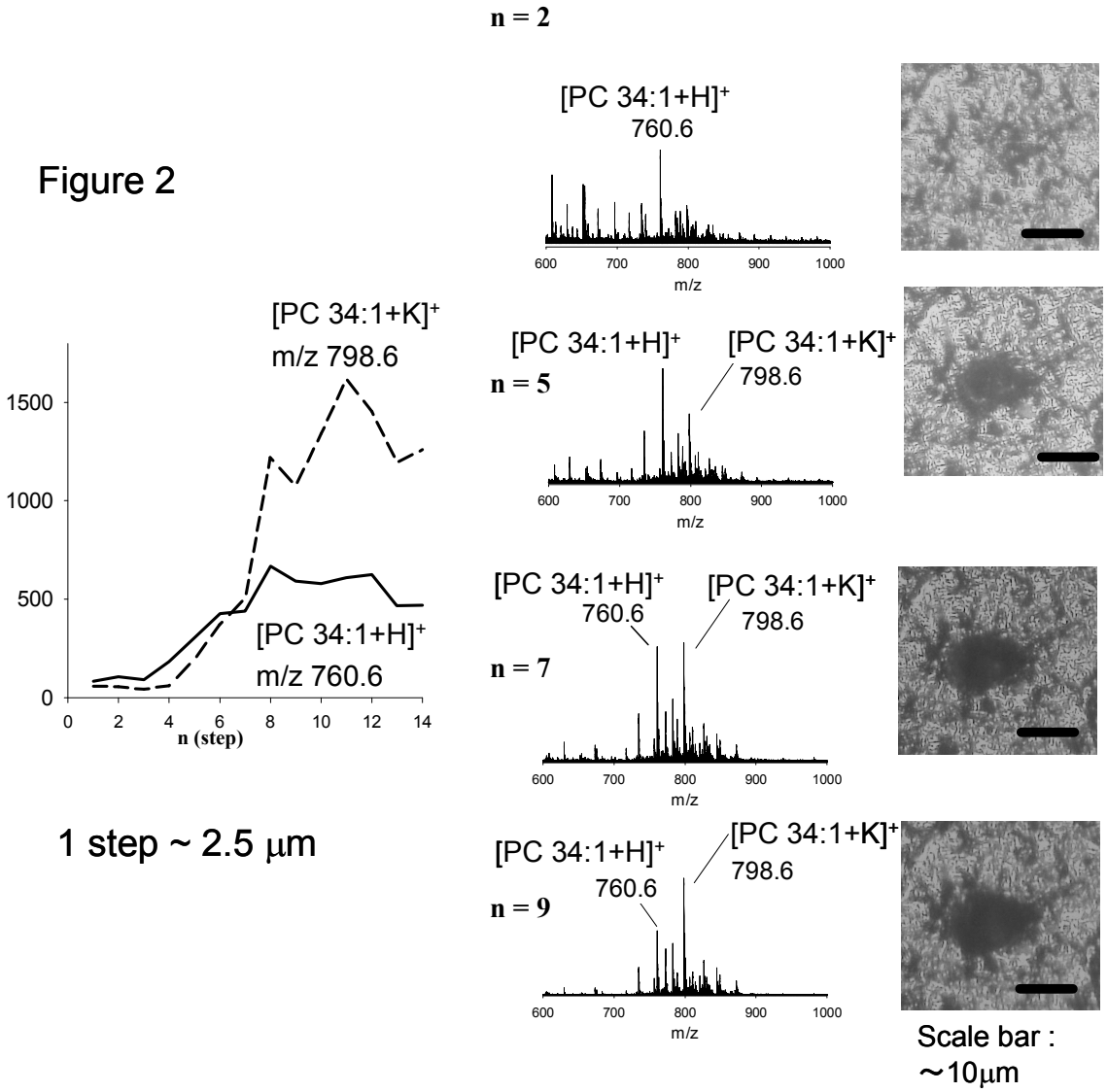

Fig. 2 Depth profile of mouse brain section analyzed by probe electrospray mass spectrometry (PESI-MS).