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Neutral Desorption Sampling of Living Objects for Rapid Analysis by Extractive Electrospray Ionization Mass Spectrometry**

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Mass spectrometry is increasingly used for research purposes and routine measurements in biology, life science, the pharmaceutical industry, and in clinic diagnosis to characterize complex samples. Usually, biological samples are mixtures supported by a biological structure such as skin, tissue, muscle, or vessels and are commonly accompanied by microorganisms. Owing to ion suppression effects, the matrix must traditionally be carefully removed, usually in a time-consuming process (e.g. extraction, separation), before mass spectrometric analysis. This processing renders high-throughput analysis difficult. Remarkable efforts to enhance analysis throughput of mass spectrometry were made by Cooks and co-workers[1] with the invention of desorption electrospray ionization (DESI). DESI, as well as DART (direct analysis in real time),[2] directly analyzes complex solid surfaces[3,4] with minimal sample pretreatment, although the sample can also be a liquid such as urine.[5] In DESI, ion suppression is reduced, because analyte ions are created by reactive collisions between the analyte molecules supported by a solid substrate and the primary ions generated by an electrospray. Recently, Chen et al.[6] demonstrated that liquid samples can be analyzed directly in real time using extractive electrospray ionization (EESI), in which ions of analytes are created by charge-transfer collisions between the primary ion cloud generated in an electrospray and a neutral sample plume generated by a separate spray beam. Thus, ion suppression is significantly reduced in EESI by dispersing the sample over a relatively wide space. Previous studies show that EESI tolerates complex matrixes.[6–10]

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Herein, we report a novel method using a neutral gas stream to sample the surface of solid biological objects for in vivo EESI mass spectrometric analysis of living matter without sample pretreatment. The sampling process is totally separated from the ionization process, which results in rapid in vivo analysis of biological surfaces with reduced ion suppression and without chemical contamination.

Experiments were carried out using a commercial quadrupole time-of-flight (QTOF) mass spectrometer in which EESI was successfully implemented without any hardware modification.[9,10] The concept of neutral desorption sampling is schematically shown in Figure 1. This sampling technique

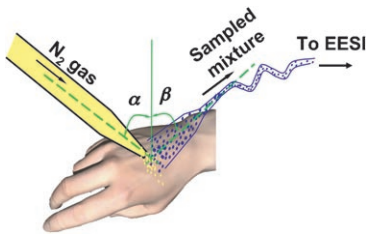

Figure 1. Schematic diagram of neutral gas desorption sampling coupled to EESI for rapid analysis of biosamples. For example, human skin, frozen meat, and plant tissue can be directly sampled in vivo for rapid chemical fingerprinting neither requiring sample pretreatment nor causing toxic chemical contamination.

separates the ionization process from the sampling process in time and space, which minimizes ion suppression. Neutral analytes can be transported over a distance longer than 1.2 m with sufficient transfer efficiency for remote analysis. This feature is of practical relevance for multiple applications; especially in cases where the sample is not accessible because of extreme environmental conditions (e.g. high or low temperature, biohazards, or radioactivity). Furthermore, the neutral gas desorption sampling technique is a gentle method that protects the biological objects from destruction, invasion, or interaction with reagents such as charged particles. Thus, no chemical contamination occurs during the sampling process, which is a highly desirable feature for applications where the native physiological or pathological status of animals or plants should be diagnosed in vivo.

The first example is a demonstration of in vivo human skin analysis. Mass spectral fingerprints of skin were recorded directly from the hand of a healthy volunteer (38-year-old male, regular smoker, 12–15 cigarettes per day) before and after he drank three cups of black coffee. The peak at m/z 163 in the mass spectral fingerprint (Figure 2) is assigned to protonated nicotine ($M_W = 162$). No signal at m/z 195 was

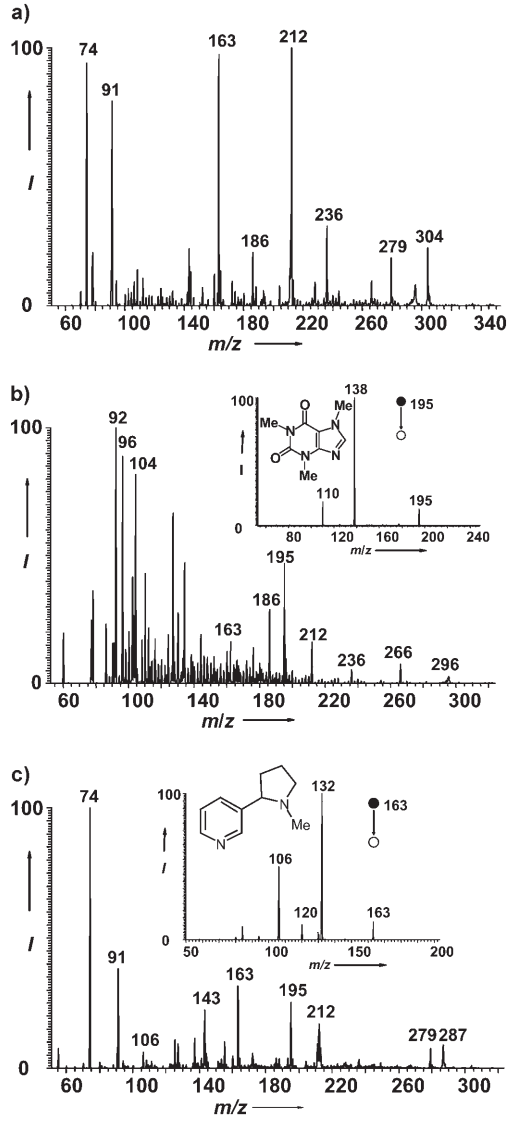

Figure 2. Dynamic mass spectral fingerprints obtained in EESI-QTOF-MS using the neutral gas desorption sampling technique shown in Figure 1. a) Metabolic fingerprints recorded directly from human hand skin before coffee consumption; b) metabolic fingerprints recorded directly from the same human hand skin area 30 min after coffee consumption; c) metabolic fingerprints recorded directly from the same human hand skin area 60 min after coffee consumption. Insets in (b) and (c) show CID spectra of caffeine and nicotine, respectively.

detected from the skin before the coffee was consumed (Figure 2a). The mass spectral fingerprint changed significantly 30 min after coffee consumption. For example, a peak at m/z 195 (Figure 2b), arising from protonated caffeine ($M_W = 194$), shows up with a higher abundance than that of nicotine (m/z 163). The collision-induced dissociation (CID) spectra of m/z 163 (inset of Figure 2c) and m/z 195 (inset of Figure 2b) are identical to those obtained using authentic compounds. After 60 min, the mass spectral pattern is further changed, and nicotine (m/z 163) and caffeine (m/z 195) are detected simultaneously with similar abundances (Figure 2c). The sampling area on the hand (Figure 1) was cleaned prior to experiments using an ethanol/water solution (4:1; medical-grade ethanol) so that chemicals accumulated from the environment are not likely to be detected in the experiments. Clearly, the data show that the mass spectral patterns obtained using the neutral gas desorption sampling technique coupled to EESI vary with the amount of coffee consumed. Besides nicotine and caffeine, which are clearly linked to the consumption of cigarettes and coffee, most peaks detected in Figure 2 are probably associated with metabolism of the human body.

Similarly, metabolites in different areas of the human skin can be fingerprinted in vivo using this technique. The spectral fingerprint of human skin (healthy 33-year-old male, non-smoker) sampled from different areas, such as forehead, abdomen, and foot, was found to be different in terms of peak density and signal intensity. For example, the signal intensity of m/z 181, detected from forehead skin, exhibits the highest abundance among the samples tested (see Table S1 in the Supporting Information). The peak at m/z 181 recorded from skin of the head was identified as protonated glucose ($M_W = 180$), which generated fragments of m/z 163, 151, and 105 by the loss of water, HCHO, and $C_2H_4O_3$, respectively, in CID experiments. Fewer peaks were recorded from the foot skin sample, probably because foot skin is much harder than other skins tested. Thus, fewer metabolites can be excreted from the foot skin. The different mass spectral fingerprints recorded from the epidermis might give hints about the metabolism of underlying local organs.

This method is also useful to detect ambient compounds accumulated on skin. For example, after exposure to air containing traces of cyclotrimethylenetrinitramine (RDX, a typical explosive, $M_W = 222$) and methylphosphonic acid dimethyl ester (DMMP, a well-known chemical warfare agent simulant, $M_W = 124$), sub-picogram quantities of RDX and DMMP can be detected as protonated molecules from human skin. If the skin contains sodium salts, DMMP can be detected as both protonated molecules (m/z 125) and ionic sodium clusters (m/z 147). These data are in good agreement with previous studies.[1] The detection of RDX and DMMP was confirmed with authentic compounds.

Another important application of this method is online food quality monitoring, which is of paramount importance to human health,[11,12] the global economy, and homeland security[13] (see the Supporting Information for details) but challenges analytical science in terms of sensitivity, throughput, and feasibility for online monitoring.[14,15] Successful detection of the freshness of frozen fish (−20 °C) is demonstrated based on the metabolic fingerprints recorded using the technique reported herein. Figure 3 shows different mass spectral fingerprints observed in artificially spoiled fish meat. Peaks of interest, such as m/z 122, 88, 116, are tentatively assigned from their MS/MS spectra (see Figure S2 in the Supporting Information) to be compounds containing $-NH_2$ or −SH groups (see the Supporting Information for MS/MS data). Based on comparison with reference compounds (see Table S2 in the Supporting Information), many peaks are identified as biogenic amines, which are biomarkers for microorganisms growing on meat.[16–19] For example, trimethylamine ($M_W = 59$, detected in protonated form at m/z 60) shows a strong signal, while dimethylamine ($M_W = 45$) and

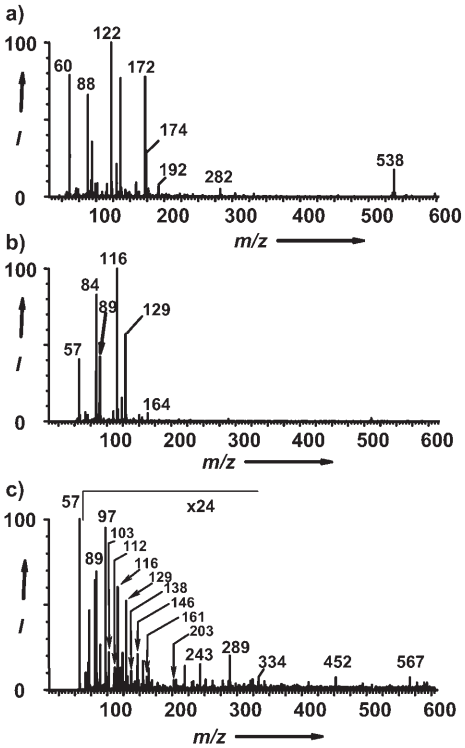

Figure 3. Desorption extractive electrospray ionization mass spectra of fish meat at different stages: a) frozen fish not exposed to room temperature; b) frozen fish after exposure to room temperature (22 °C) for one day; c) frozen fish after exposure to room temperature for two days. Numerous peaks were detected in the mass range from *m*/*z* 50–800. Tyramine ($M_W = 137$), tryptamine ($M_W = 160$), and spermine ($M_W = 202$) were not detected after exposure to room temperature for less than two days, but they were detected as protonated molecules with relatively low intensities in samples after exposure for two days.

dimethylacetylamine ($M_W = 73$) give relatively low intensities (Figure 3a) in the freshest sample. All the alkylamines except trimethylamine are undetectable in fish samples after exposure to room temperature. Intensity changes were also found for the peaks at *m*/*z* 122 and *m*/*z* 88, probably because of the metabolic dynamics of the microorganisms growing on the spoiling fish sample.[18,20,21] We could never smell a typical fresh fish odor, possibly because the fresh fish sample was not truly fresh.[22] An unknown peak at *m*/*z* 57 (see MS/MS data in the Supporting Information) is quite strong in the heavily spoiled sample, while it was not detected in the fresh fish. Histamine, a typical molecular marker for various spoiled foods,[16,18,19,21] is the main toxin involved in histamine fish poisoning[23] and other biochemical disorders.[24] Experimentally, histamine (*m*/*z* 112) and putrescine (*m*/*z* 89) were detected in the fish exposed to room temperature for one day. All the typical biogenic amines including putrescine, cadaverine, histamine, tyramine ($M_W = 137$), spermidine ($M_W = 145$), tryptamine ($M_W = 160$), and spermine ($M_W = 202$) were detected, as protonated molecules, with increased intensities in the fish exposed to room temperature for two days (Figure 3c). The multiple peaks shown in the mass spectral fingerprints of spoiled fish might give useful hints to understanding why histamine consumed in spoiled fish is more toxic than pure histamine taken orally.[23] For histamine, the detection limit was found to be 10 fg cm^{-2} (signal-to-noise ratio S/N = 3) on the surface of frozen meat and to be 0.03 wt % (S/N = 8) in meatloaf. Samples can be monitored in an online high-throughput fashion (see the Supporting Information).

Finally, experiments were performed to obtain data from plants. For example, several peaks distributed in the mass range *m*/*z* 100–600 show up in the mass spectral fingerprints (Figure 4a) recorded from green iceberg lettuce leaves.

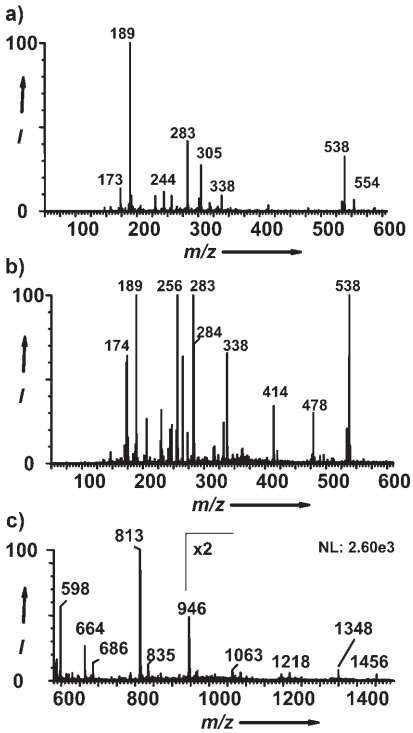

Figure 4. Extractive electrospray ionization mass spectral fingerprints of iceberg lettuce samples: a) fresh, green iceberg lettuce leaves; b) fresh iceberg lettuce leaves contaminated by *E. coli* strain TG1. c) Multiple peaks detected in the relatively high-mass range from sample (b). NL = normalization level.

Similar spectral patterns were obtained using different individual leaves. In contrast, more peaks (e.g. *m*/*z* 174, 256, 414) were detected from lettuce leaves contaminated by *E. coli* bacteria (Figure 4b). This finding indicates that the new peaks are associated with bacterial growth. Ions of *m*/*z* 538, probably a metabolite, were commonly detected on the fresh fish sample, the foot skin sample, and the iceberg lettuce samples. However, these ions were not detected in the spoiled fish samples, thus indicating that they are not due to an artefact. In the absence of comparison with an authentic compound, it is difficult to speculate as to the molecular structure of the compund, although it might be associated with metabolism of the living organisms on the samples. Multiple small peaks were detected in a relatively high-mass range (above 1000 Da) from the iceberg lettuce samples contaminated by *E. coli* (Figure 4c). These high-mass peaks might result from peptides originating from the growth of

E. coli strains. The data show that neutral gas desorption sampling is able to sample compounds up to fairly high molecular weight (above 1000 Da) directly from biological samples. The high-molecular-weight species and other chemicals like nicotine, caffeine, and histamine are typical semivolatile compounds that are not likely to be sampled directly in vapor form. Therefore, the experimental results support the notion that efficient neutral desorption and transportation takes place before the EESI process.

In summary, we report a simple approach that allows investigation of virtually any kind of sample by a gentle stream of nitrogen or other gas. This sampling technique is coupled to an EESI source implemented in front of a commercial QTOF mass spectrometer for direct analysis of various biological samples without any chemical contamination or sample pretreatment. Ion formation in this method does not suffer from matrix effects owing to the separation of the desorption and ionization processes. The overall result is a very efficient process capable of achieving very low detection limits for complex samples. The mass spectral fingerprints display metabolites originating either from growing microorganisms or from the sample itself and thus produce a molecular signature for a wide variety of biological samples. This novel metabolomics-based strategy represents a "green" procedure for fast chemical characterization of biological objects.

Experimental Section

Experiments were carried out using a commercial quadrupole time-of-flight (QTOF) mass spectrometer in which EESI was successfully implemented without any hardware modification.[9,10] To minimize the ion suppression effect, analytes were sampled from a biological surface by a gas stream (e.g. dry nitrogen gas, 1–3 $L\,min^{-1}$) to form an aerosol gas phase mixture, which was then transported through a teflon tube (5 mm inside diameter, 1.2 m in length) to reach the EESI source, where the molecular analytes were ionized by ESI spraying of aqueous acetic acid solution (10 vol %) for mass analysis (shown in Figure 1). To facilitate the neutral desorption sampling process, a sharp jet (i.d. 1 mm) was formed at the end of the gas line so that a high gas speed (about 10 $m\,s^{-1}$) was created. A V-shaped collecting tube (i.d. 10 mm) was mounted in front of the teflon tube so that more aerosol could be collected. The angles between the axes of the desorption gas flow and the collecting tube were set such that $\alpha = 45$–$70°$, $\beta = 60$–$45°$ (Figure 1). The distance between the gas jet tip and the surface was 2–10 mm.

Target compounds such as RDX and DMMP were sprayed into the ambient air as aqueous solutions (10 ppt, 2 mL). A hand with clean skin was exposed to the aerosol to passively collect the target compounds. Then the hand skin was sampled directly without further treatment. EESI-TOF mass analysis is described elsewhere.[9,10]

The fish sample, cut into 3–5 pieces (25–100 g each), was kept frozen at −20 °C after exposure to air at room temperature (22 °C) for 0, 1, or 2 days to generate different spoilage stages. All meat samples were maintained at −20 °C during measurements. All types of food samples were bought from local stores.

E. coli TG1 strains, a gift from Dr. Chunmei Li (Institute of Molecular Systems Biology, ETH Zurich, Switzerland), were centrifuged at 1000 rpm and flushed with water three times to get rid of the medium. Iceberg lettuce was separated into two groups (150 g each). The control group was kept at 5 °C to keep it fresh before use. The experimental group, sprayed with *E. coli* strains (5 mL), was kept at 30 °C for 6 h before use. All the iceberg lettuce samples were still wet on their surfaces and were directly used without further treatment.

All chemical reagents were bought from Fluka (Buchs, Switzerland) with the highest purity available for direct use without any further purification. Deionized water was provided by ETH chemistry facilities.

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