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Gold nanoparticle-enhanced target for MS analysis and imaging of harmful compounds in plant, animal tissue and on fingerprint

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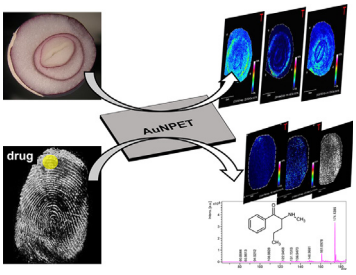
HIGHLIGHTS

- First use of gold nanoparticle method for detection and imaging of designer drug.
- Co-localization of drug and fingerprint endogenous lipids is shown.
- AuNPET aided analysis of very complicated biological mixtures is shown.
- Harmful and cancerogenic compounds in onion and liver were detected.

GRAPHICAL ABSTRACT



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ABSTRACT

Gold nanoparticle-enhanced target (AuNPET) was used for detailed investigation of various materials of biological origin – human fingerprint, onion bulb and chicken liver. Analysis of these objects was focused on toxic and harmful compounds – designer drug containing pentedrone, diphenylamine in onion and potentially cancerogenic metronidazole antibiotic in liver. Detection of large quantity of endogenous compounds from mentioned objects is also shown. Most of analyzed compounds were also localized with MS imaging and relationship between their function and location was discussed. Detected compounds belong to a very wide range of chemical compounds such as saccharides, ionic and non-ionic glycerides, amino acids, fatty acids, sulfides, sulfoxides, phenols *etc.* Fingerprint experiments demonstrate application of AuNPET for detection, structure confirmation and also co-localization of drug with ridge patterns proving person-drug contact.

1. Introduction

The use of gold nanostructures in laser desorption/ionization (LDI) mass spectrometry (MS) of low molecular weight compounds was covered recently in an excellent review by Bergquist and coworkers [1]. Gold nanostructures have been used more frequently since publication of the pioneering work by Russell [2], who presented methods of preparation of size-controlled gold nanostructures. Currently, a large increase in the number of reports in which authors make use of gold structures is observed [3]. Gold nanostructures have been prepared by a wide range of methods, but most often by gold(III) reduction with citrate or $NaBH_4$ [4–6]. The use of each of the two reducing agents mentioned may result in numerous interfering peaks [2,3,7,8]. To the few methods allowing chemical background reduction belong laser ablation methods,

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providing chemical-free AuNPs in solution (LASiS) [9] and our gold nanoparticle-enhanced target AuNPET presented in year 2015 [10].

Last two years brought few very interesting reports on AuNPs applications in LDI MS. Recently, a method of iodide anions determination in urine with the use of gold nanoparticle-modified cellulose membranes was described [11]. Unique gold hybrid nanoflowers on manganese oxide (Au@MnO) were found to be an efficient matrix for LDI-MS determination of adenosine triphosphate [12]. Zeolite loaded with gold nanoparticles was shown to ionize low molecular weight compounds, such as amino acids, but also non-ionic urea [13]. Colaianni et al. [14] tested methods for purification of gold nanowires for efficient determination of a model 1 kDa peptide, and similar objects – plasma peptides, were investigated on porous silicon–gold plasmonic nanostructures by Wu and co-workers [15]. Moreover, our group has shown recently application of AuNPET for high-resolution analysis and imaging of fingerprint for endo- and exogenous compounds [10].

In this work, examples of application of AuNPET for analysis and MS imaging of important biological objects of varying character such as plant, animal tissue and also fingerprint are provided with focus on xenobiotic and/or endogenous toxic and harmful substances.

2. Materials and methods

2.1. Materials and equipment

AuNPET target plate preparation was described in our recent work [10]. All solvents were of HPLC quality. Optical photographs were made with the use of an Olympus SZ10 microscope equipped with an 8 MPix Olympus digital camera. Chicken liver, red onion (*Allium cepa L. 'Red Baron')* and designer drug ("Cząstka Boga") were obtained from local shops (Rzeszow, Poland). Image F in Fig. 1 was made by superimposing of three images of *m/z* 312.2327, 211.1211 and 192.1388, the resulting image's blue channel was then converted to grayscale (24 bit) and saved as lossless TIFF file. Image B in Fig. 2 was made by superposition of C–K images from the same Figure with conversion to grayscale. For both superimposed images adjustments of contrast and brightness were applied for maximum visibility of details. No image modification was made to any other ion image.

2.2. LDI MS experiments

LDI-ToF mass spectrometry experiments were performed using Bruker Autoflex Speed time-of-flight mass spectrometer equipped with a SmartBeam II laser (355 nm). Laser impulse energy was approx. 100–150 μJ, laser repetition rate – 1000 Hz. Suppression was turned on typically for ions of *m/z* lower than 60. The first accelerating voltage was held at 19 kV, and the second ion source voltage at 16.7 kV. Reflector voltages used were 21 kV (the first) and 9.55 kV (the second). The data was calibrated and analyzed with FlexAnalysis (version 3.3) using centroid calibration model. Mass calibration (typically enhanced cubic calibration based on 5–10 points) was performed using internal standards (gold ions and clusters from Au^+ to Au_{10}^+ and also $[Na_2Cl]^+$, $[NaKCl]^+$ and $[K_2Cl]^+$ depending on *m/z* range). Compound search was aided by Lipidomics Gateway (http://www.lipidmaps.org) and Human Metabolome Database (HMDB, http://www.hmdb.ca).

2.2.1. MS analysis of onion water extract on AuNPET

Internal fragment of *A. cepa* (48 mg) was crushed in Eppendorf vial and mixed with 1 ml of water, vortexed for 5 min and inserted into ultrasonic bath (5 min, RT). One microliter of extract was placed directly on AuNPET, air dried, and measured within 60–1500 *m/z* range (10500 laser shots).

2.2.2. MS analysis of onion acetone extract on AuNPET

Internal fragment of *A. cepa* (78 mg) was crushed in Eppendorf vial and mixed with 1 ml of acetone, vortexed for 5 min and inserted into ultrasonic bath (5 min, RT). One microliter of extract was placed directly on AuNPET, air dried, and measured within 60–1500 *m/z* range (25500 laser shots).

2.2.3. MS analysis of liver acetone extract on AuNPET

Internal fragment of chicken liver (~2.5 g) was inserted into glass flask with 2 ml of acetone and stirred (700 rpm) overnight. After centrifugation, 0.5 μL of extract was placed directly on AuNPET, air dried, and measured within 60–1000 *m/z* range (50000 laser shots, suppression of ions under *m/z* 55). Additional spectrum in 600–2000 *m/z* range (35500 laser shots; suppression of ions under *m/z* 590) was also made.

2.2.4. MS analysis of liver water extract on AuNPET

Internal fragment of chicken liver (311 mg) was inserted into glass flask with 2 ml of water and stirred (700 rpm) overnight. After centrifugation, extract (1 μL) was placed directly on AuNPET, air dried, and measured within 60–1000 *m/z* range (20000 laser shots).

2.2.5. MS analysis of designer drug on AuNPET

Drug stock solution (1.18 mg/ml of water) was placed directly on AuNPET, air dried, and measured within 60–1000 *m/z* range (1500 laser shots). Additionally, 100-times diluted stock drug solution was also made and measured. LIFT MS/MS spectra were also made for drug-sodium adduct (5000 laser shots, 40–230 *m/z* range).

2.3. LDI MS imaging experiments

Measurements were performed using a Bruker Autoflex Speed time-of-flight mass spectrometer in positive-ion reflectron mode. The apparatus was equipped with a SmartBeam II 1000 Hz 355 nm laser. Laser impulse energy was approximately 100–190 μJ, laser repetition rate was 1000 Hz, and deflection was set on *m/z* lower than 60 Da. The *m/z* range was 60–1000 for the liver and drug experiments and 60–1500 for onion experiment. The experiments were made with 500 laser shots per individual spot with a default random walk applied (FlexImaging 4.0).

All spectra were calibrated with the use of gold ions (Au^+ to Au_7^+) and also $[Na_2Cl]^+$, $[NaKCl]^+$ and $[K_2Cl]^+$ depending on *m/z* range. The first accelerating voltage was held at 19 kV, and the second ion source voltage was held at 16.7 kV. Reflector voltages used were 21 kV (the first) and 9.55 kV (the second). All of the shown imaging pictures are for ±0.05% *m/z* window. TIC normalization was used with all results shown.

2.3.1. MS imaging of Allium cepa tissue

Onion of typical size (*ca.* 60 mm diameter) was washed with deionized water and few external layers were carefully removed. Internal material (*ca.* 20–25 mm diameter) was then cut perpendicularly to apex-root axis with razor blade. Onion cross-section was then immediately touched to the cellulose filter paper (3 times) to remove excess of liquid material and touched to AuNPET plate with light pressure for 3 s and removed. Imaging experiment was made at 200 × 200 μm resolution.

2.3.2. MS imaging of designer drug on finger

Designer drug in form of fine powder was placed (*ca.* 1 mg) on small section of finger for 5 s and wiped off with cellulose cleaning paper (2 times) until no powder was visible. Finger was then