Quantitative Mass Spectrometry Imaging (QMSI) is used to evaluate the amount of a large molecule (higher than 3000 Da) within tissue. Methodology of quantification using a «pseudo internal standard» covering the sample is explained ("Modified Standard" Approach) and applied to the example of mouse insulin assessment in pancreas tissue. To ensure fast data treatment Quantinetix™ software is used in order to calculate the amount of target molecule.

Introduction

Mass Spectrometry Imaging (MSI) has become a common technique to detect the localization of molecules directly on the surface of biological tissues. Recently, numerous studies have dealt with the growing interest in combining quantitative and distribution analyses using MSI [1,2]. Moreover, new methodologies have been developed to address the limitations of quantification using MSI i.e. reproducibility, tissue-specific ion suppression and molecule specific ionization yield. One of them is the "Modified Standard" approach. It uses a labeled, an isotope or an analogue molecule with similar properties as the target molecule to normalize its signal on tissue or on the slide. In combinaison with a calibration curve obtained using same conditions, we are able to quantify the amount of molecules within tissue while taking into account QMSI limiting factors. This application may play a significant role in early phases of pharmaceutical discovery to evaluate small molecule concentration, notably drugs. Therapeutic peptides are a new and fast growing field in which MS imaging could play a role. The developed approach uses Insulin analogue peptide in order to normalize registered images and quantify endogenous insulin in pancreas and especially in Langerhans Islets.

Experimental

Pancreatic fasted mouse tissue sections (in triplicate) were carried out with a Microm cryostat HM560 (Thermo Scientific, USA), at 10 µm thickness. All sections were mounted on conductive ITO glass slides, and then dried. A dilution range of
Figure 1  
Global workflow of the "Modified Standard" Approach for QMSI.

- **Matrix**
- **Modified Standard**
- **MALDI MS image**

**Figure 2**  
MS image of the dilution range and corresponding calibration curve (image and data from Quantinetix™)

**Figure 3**  
Methylene blue staining of three serials pancreas sections, distribution of insulin in these samples by MSI, and the quantification of insulin in Langerhans islets and whole pancreas tissue (image and data from Quantinetix™).
human insulin (6 droplets of 1 µL between 0 and 50 µM) dissolved in water (HPLC grade) was deposited near tissue cryosection on the ITO slide. DHB at 40 mg/mL in methanol/water/trifluoroacetic acid (MeOH/H2O/TFA, 50/50/0.1, V/V/V) was used as the matrix solution. The matrix solution was sprayed onto the pancreatic sections using the SunCollect automatic sprayer (SunChrom, Friedrichsdorf, Germany). Analogue of Human Insulin (Lantus, Sanofi) was used as “pseudo internal standard” (at 10 µM, m/z 6060) sprayed mixed with the matrix on the entire slide.

MS images were acquired with an AutoFlex speed LRF MALDI-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with a Smart beam II laser used at a repetition rate of 1000 Hz. All instrumental parameters were optimized before the imaging experiment on standard samples of human insulin at m/z 5803. Positive mass spectra were acquired within the 3000- to 15000-m/z range. The mass spectrometer was operated in the linear mode and the mass spectrum obtained for each image position corresponds to the averaged mass spectra of 1000 consecutive laser shots at the same location. Two image raster steps were selected: 150 µm for MS imaging of dilution range (∼ 5000 voxels) and 70 µm for pancreas tissue images (∼10000 voxels). Cryosections of mouse pancreas were stained with methylene blue after MSI analyses in order to finely localize Langerhans islets. Quantinetix™ (ImaBiotech) Software was used to assess Insulin level in tissue sample following “Modified Standard” approach (Calibration & Analyze view) [2].

Results

Insulin is a peptide hormone, synthesized in the pancreas by the beta cells of the islets of Langerhans. It plays a key role to regulating carbohydrate and fat metabolism in the organism. A quantitative mass spectrometry imaging methods is used to localize and quantify this peptide in pancreas tissue section. The figure 1 shows the workflow of the “Modified Standard” approach applied in our study. In this method, ion suppression effect of the tissue on the molecule signal is compensated by the use of a ratio. For one specific voxel, the intensity of the target molecule is divided by the intensity of the standard molecule. This ratio allows correlating a signal from the dilution range with a signal from the tissue itself and generates quantitative data for different histological area of the tissue or for the whole sample.

Calibration curve of human insulin molecule (m/z 5808) is calculated using imaging data as shown in figure 2. From standard dilution series and for each concentration spot (5 in this case), molecular image is constructed (left side), mean intensity ratio values are extracted and correlated to amount of drug per surface unit. For the data treatment, a mass filter window of 10 Da was selected according to the poor resolution of the linear mode of the TOF instrument (R=500). The control area (0 µM) is used to subtract noise signal from dilution range imaging data. The human insulin species exhibit a higher limit of detection (1 µM) than small molecules such as drugs or lipids (0.01µM). The calculated coefficient of calibration curve value (r²) was 0.997 which shows that a good linearity was obtained.

The molecular image of mouse insulin ion (m/z 5799) in the three adjacent sections of mouse pancreas is displayed on the figure 3. These MS images correspond to the distribution of normalized mouse insulin signal with “modified standard” and consequently show the “real” response of the molecule in tissue. Methylene blue staining is used to highlight the islets of Langerhans on tissue section (light blue region on the optical image of figure 3). We can observed that insulin is mainly localized at the level of the Langerhans islets which the site of production of the peptide. In addition, glucagon related ion (m/z 3483) was also observed on mass spectra from Langerhans islets region but was not quantified in this study. Mouse insulin amount was determined in whole pancreas tissue, at approximately 260 µg/g of tissue, but also in Langerhans islets. In this small histological region, the content of mouse insulin was significantly higher, in the mg/g of tissue level. An inter-sample mean variation of 30% was observed which is acceptable for a biological related study on tissue section using mass spectrometry imaging. These results were in agreement with previously published data [3,4] on the quantification of mouse insulin using liquid chromatography (approximately 165 µg/g of tissue for fasted mouse).
Quantitative Mass Spectrometry Imaging using “Modified Standard” approach was successfully applied to evaluate mouse insulin amount in pancreas tissue section. These results are the first example of direct quantification of endogenous insulin in Langerhans islets tissue. Moreover, the use of Quantinetix™ software allows a faster the data treatment and the generation of quantitative results. In conclusion, QMSI can give some useful and fast quantitative information about a molecule trapped in tissue which can be a small or a large compound.