Novel mass tags for the sensitive detection of steroidal ketones by MALDI MSI

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Overview

Objective: Improve MSI steroid detection sensitivity after on tissue chemical derivatization by generation of mass tags possessing optimized integrated charged absorbing modules (ICAMs).

Methods: Cholesterol-ICAM adducts were first formed to assess detection sensitivity by MALDI MS. Best ICAMs were attached to hydrazide warheads and resulting tags screened on tissue.

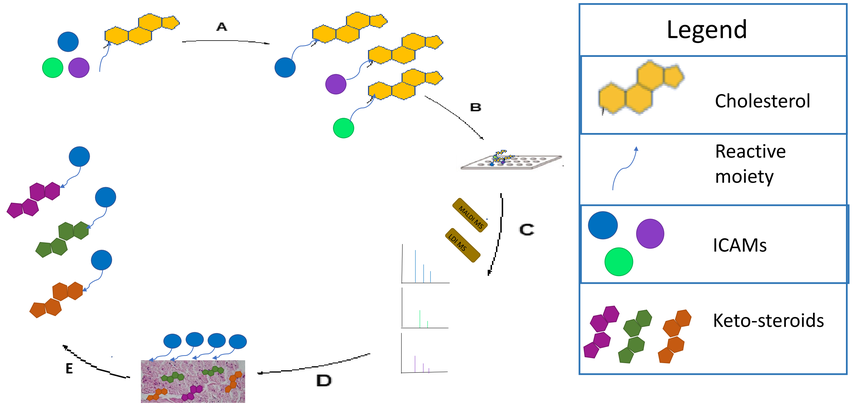
Results: Compared to Girard's reagent P standard, optimized ICAM-hydrazide tags exhibited increased MALDI MSI sensitivity for steroids on tissue.

Introduction

Steroids are a vital component of healthy metabolism. Often dysregulated in cancer and other diseases, steroids are commonly used to treat disorders such as asthma and allergies.

On-tissue chemical derivatization (OTCD) techniques in MALDI MSI are used to image compounds that cannot be detected sensitively using traditional MALDI MSI.[1] Steroids are primarily visualized after OTCD, because they suffer from both low ionization and matrix interference. Low sensitivity of detection has limited steroids imaging by MALDI MSI using Girard's reagents P and T, and 2,4-dinitrophenylhydrazine (DNPH).[2-4] Mass tags are desired with increased sensitivity for the detection of keto-steroids.

Workflow



Novel integrated charged absorbing modules (ICAMs) were identified for introduction into mass tags with ketone-specific reactive warheads. (A) ICAMs are linked to cholesterol to form stable adducts. (B, C) Adducts are screened by MALDI MS. (D, E) ICAMs from adducts detected with high sensitivity were then attached to warheads and used in mass tags for sensitive imaging of steroids by MALDI MSI using adrenal gland tissue sections.

Results

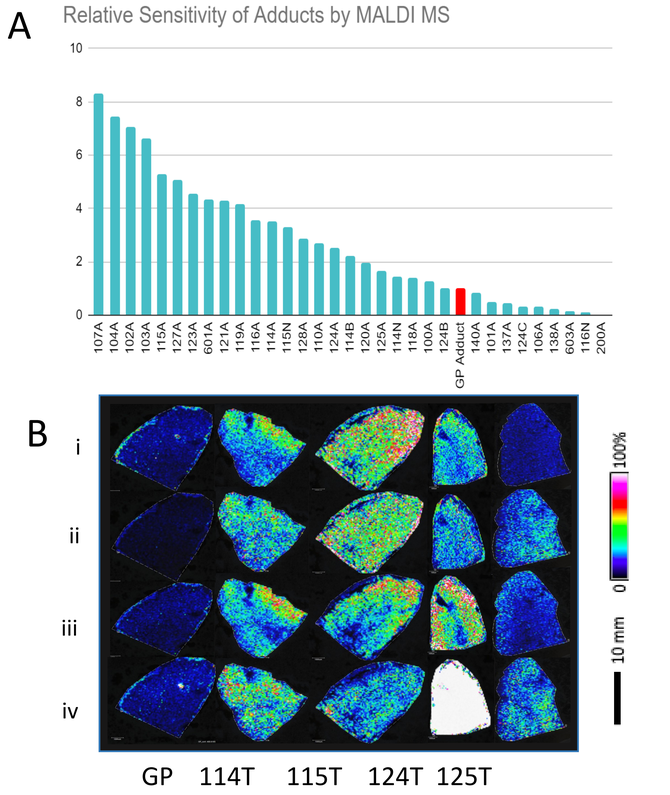


Figure 1: (A) Relative sensitivity of ICAM-cholesterol adducts with respect to Girard's Reagent P. Adducts and matrix were subsequently spotted on plate, with samples allowed to dry fully between. MALDI MS profiling of samples was performed. The average signal-to-noise ratio of three spectra were plotted. (B) ICAMs from adducts detected with high sensitivity were used as mass tags to image steroids on rabbit adrenal gland tissue sections. ICAMs were spray-deposited using an automated sprayer system. Ion images were generated by MALDI MSI at *m/z* ratios corresponding to $[Tag+Steroid-H_2O]^+$ for (i) testosterone/DHEA, (ii) progesterone, (iii) androstenedione and (iv) corticosterone.

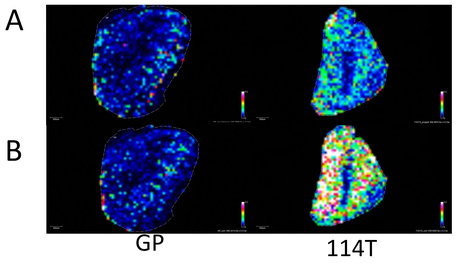


Figure 2: MALDI MSI ion images of (A) progesterone and (B) corticosterone comparing Girard's Reagent P and 114T. Tags were spray deposited prior to incubation for 1h. CHCA was spray deposited and samples were imaged at a spatial resolution of 100 µm on a MALDI-TOF MS. The images for 114T have 8.7 and 10.2-fold improvement in sensitivity with respect to Girard's Reagent P, respectively.

- Over 40 ICAM-cholesterol adducts were generated and screened by MALDI MS.
- Tags possessing ICAMs from the most sensitive adducts were tested on tissue.
- Tags with optimal ICAMs gave over 10-fold greater sensitivity for certain steroids compared to Girard's reagent P.
- Reactivity seems to be steroid specific, as evidenced by 124T, which shows a 700-fold improvement in sensitivity for corticosterone and only 3.7-fold for testosterone when compared to Girard's reagent P.

Conclusions

- Novel ICAM reactive mass tags allow rapid and sensitive screening of ketone steroids in tissue sections by MALDI MS.
- Optimized ICAM tags demonstrated a sharp increase in sensitivity when compared to Girard's reagent P.
- The sensitivity of some tags with optimized ICAM was steroid specific. Others ICAM-derivatized tags offered improved detection across all steroids.

Acknowledgements

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References

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