MALDI-IT-TOF-MS/MS analysis of cuticular lipids from *Anopheles gambiae* mosquitoes

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Overview

➢ MALDI-MS^n was applied to extracted lipid/hydrocarbons to identify the features which were previously shown to differentiate *Anopheles gambiae* mosquitoes based on their age, sex, and mating status.[1]

➢ Individual ions associated with signal clusters (to differentiate different cohorts- male vs. female, young vs. old; mated vs. unmated) were isolated and fragmented for qualitative analysis.

Introduction

Cuticular lipids/hydrocarbons play an essential role in the recognition processes of insects. Gender, age, and mating status correlate with the changes of the lipid profile of cuticular lipids of mosquitoes, and *Anopheles gambiae* mosquitoes are the primary malaria-carrying species in sub Saharan-Africa. Rapid characterization of the cuticular lipid composition would possibly allow improved risk management and reveal effective ways to monitor malaria control efforts. MALDI-MS has been successfully applied for differentiation of cuticular hydrocarbon and lipids between different cohorts of mosquitoes. In this work, tandem mass spectrometry in an ion trap - time-of-flight MALDI-MS instrument was used to interrogate and assign specific lipids/hydrocarbons responsible for differentiation of cohorts based on age, sex and mating status.

Methods

❑ Long chain hydrocarbons and lipids from the wax layer on the insect cuticle of laboratory strain *A. gambiae* G3 mosquitoes were extracted using chloroform. One mosquito was used per extraction. Previously,[1] 3 mosquitoes were needed for good signal quality.

❑ Silver nitrate was used as a cationization reagent, in combination with a 2,5-dihydroxybenzoic acid (DHB) matrix. In small volumes, a lipid extract was mixed with matrix, $AgNO_3$, and squalene (internal standard).

❑ One microlitre of sample was spotted on a steel target plate in triplicate and air dried. MALDI-MS was performed with a Shimadzu AXIMA Resonance MALDI-IT-TOF-MS in the positive ionization mode.

Results

➢MALDI-IT-TOF-MS method was optimized to yield high intensity spectra of lipids/hydrocarbons from the cuticle of a single mosquito.

➢In young vs. old, male vs. female, and virgin vs. mated mosquitoes, quantitative differences could be visually observed between the spectral profiles.

➢Signals over an increased mass range (up to m/z 1200) correspond roughly to C90-100 compounds and a greater number of features to potentially discriminate the cohorts.

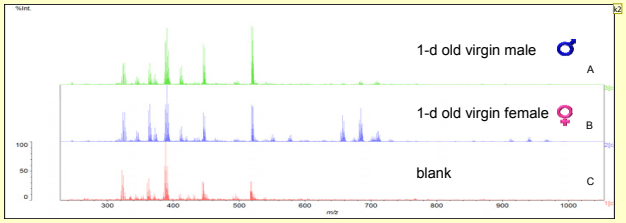


Fig. 1. MALDI spectra of cuticular lipid of one day old virgin (A) male mosquitoes compared to (B) one day old Virgin female mosquitoes and (C) blank with DHB as matrix and $AgNO_3$ as cationization reagent.

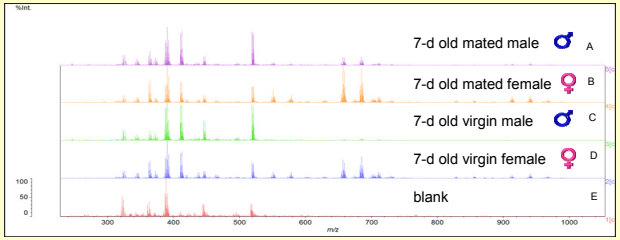


Fig. 2. MALDI spectra of cuticular lipid of seven day old mated (A) and virgin (C) male mosquitoes compared to seven day old mated (B) and virgin (D) female and (E) blank with DHB as matrix and $AgNO_3$ as cationization reagent.

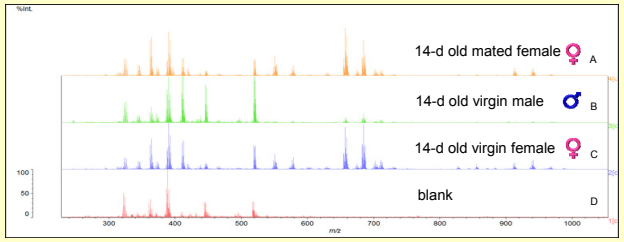
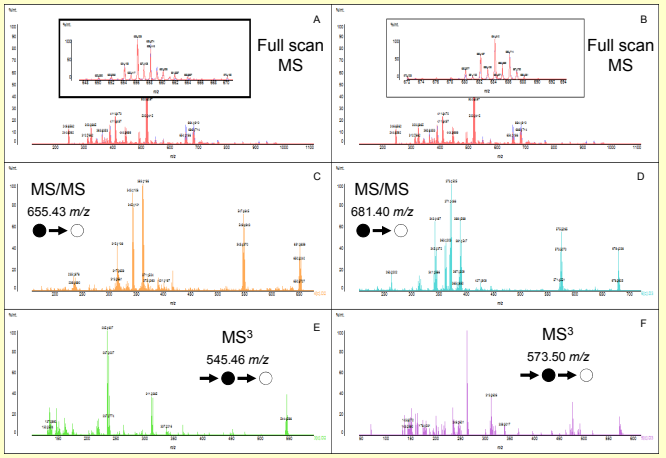


Fig. 3. MALDI spectra of cuticular lipid of fourteen day old mated (A) and virgin (C) female mosquitoes compared to fourteen day old virgin (B) male and (D) blank with DHB as matrix and $AgNO_3$ as cationization reagent.

➢Based on MS^n spectral patterns, it now seems clear that isolated signals for higher molecular weight signals (above 500 m/z) are not predominantly hydrocarbon in nature, but rather are long-chain complex lipids.



MALDI spectra of cuticular lipid of mosquitoes –The ion cluster corresponding to m/z 650-658 is shown at inset (A) and ion cluster corresponding to m/z 680-688 is shown at inset (B). (C) MS/MS of the ion at m/z 655.43; (E) MS^3 of the ion at m/z 545.46; (D) MS/MS of the ion at m/z 681.40; (F) MS^3 of the ion at m/z 573.50

Conclusions

➢Mass differences from MS/MS and MS^3 fragment ion spectra were not indiciative of hydrocarbon fragmentation; it is more plausible to attribute extracted cuticular compounds to complex lipid/phospholipid variants. Silver ion cationization confounds interpretation of fragment spectra, but future work will focus on elucidation of specific lipid identities associated with observed cuticular extract signals.

➢For some compounds the representative spectra showed some notable quantitative differences between different cohorts. As in previous work, rigorous multivariable statistical analysis (e.g. PCA and Feature Selection algorithms) will be used to characterize the differentiation power of the method.

➢The ability to perform age grading and the identity of discriminating lipids/hydrocarbons hold promise for future proposed malaria risk assessment and abatement strategies.

References

1. Suarez, E.; Nguyen, H. P.; Ortiz, I. P.; Lee, K. J.; Kim, S.B.; Krzywinski, J.; Schug, K. A. Matrix-assisted laser desorption/ionization-mass spectrometry of cuticular lipid profiles can differentiate sex, age, and mating status of *Anopheles gambiae* mosquitoes. *Anal. Chim. Acta.* **2011**, *706*, 157– 163.

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k2 I tink it would be better to put labels of the different groups directly on the panels, rather than in the captions. This would make it easier to read.
kschug, 5/8/2012