Selective gas-phase Schiff base formation of phosphatidylserine lipids in imaging mass spectrometry using charge inversion ion/ion reactions

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OVERVIEW

- **Purpose:** To separate isobaric phospholipids in imaging mass spectrometry (IMS) experiments.
- **Approach:** Charge inversion ion/ion reactions were used to selectively react with PS (but not PE) lipids using gas-phase Schiff base chemistry.
- **Results:** The differentiation of isobaric $PS_{18:0/16:0}$(*m/z* 762.528) and $PE_{16:0/22:6}$(*m/z* 762.507) is demonstrated.
- **Significance:** Gas-phase derivatization represents a rapid and selective method to differentiate compounds based on chemical reactivity without the need for chromatography or sample manipulation.

INTRODUCTION

Isobaric compounds are often unresolved in many –omics workflows **(Figure 1).** The confluence of ions with similar *m/z* values can lead to inaccurate depictions of molecular distributions in IMS experiments.

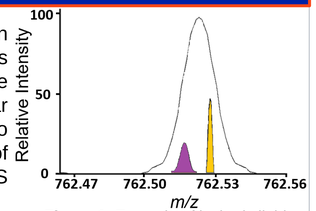

Figure 1. Example of isobaric lipids.

Gas-phase Charge Inversion Ion/ion Reactions

$$[\text{phospholipid - H}]^{1-} + [\text{Reagent}]^{2+} \rightarrow [\text{phospholipid - H + reagent}]^{1+}$$

Schiff base (Δ) *or* no Schiff base (Δ)

Charge inversion ion/ion reactions are rapid, selective, and efficient, making them ideally suited for use during IMS experiments. These reactions are performed in the gas-phase at every pixel during the imaging experiment. Rather than fractionation on the basis of molecular weight, this approach allows for separation based on different chemical reactivities.

Phospholipid Structure

Phospholipids contain a diversity of head groups. PE and PS lipids uniquely have an amino group that can potentially be targeted via Schiff base chemistry. However, PS (not PE) lipids have an additional carboxyl group, which provides the necessary H to drive water loss and complete Schiff base formation, so Schiff base chemistry will be unique to PS (and not PE).

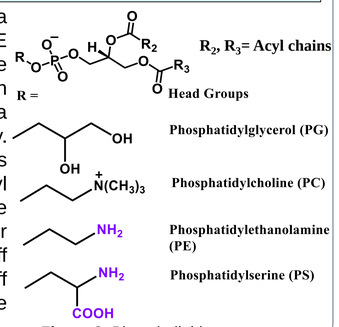

Figure 2. Phospholipid structures.

RESULTS

Schiff Base Chemistry and Instrumental Setup

- The N,N,N',N'-tetramethyl-N,N'-bis(6-oxohexyl)hexane-1,6-diaminium (TMODA) reagent has been synthesized (**Figure 3**)
- The charge inversion reaction mechanism between TMODA and PS is drawn in **Figure 4**.
- The gas-phase reactions are performed on a modified FT-ICR MS. Phospholipid anions (lipid standards or from tissue) generated via MALDI and doubly charged TMODA cations generated via ESI are sequentially isolated using the quadrupole mass filter (QMF) and injected into the hexapole reaction cell. The product ions are then transferred to the ICR cell where they are subjected to sustained off-resonance collision induced dissociation (SORI CID) and then mass analyzed (**Figure 5**).

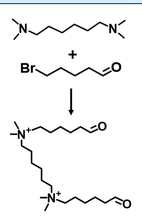

Figure 3. TMODA reagent synthesis.

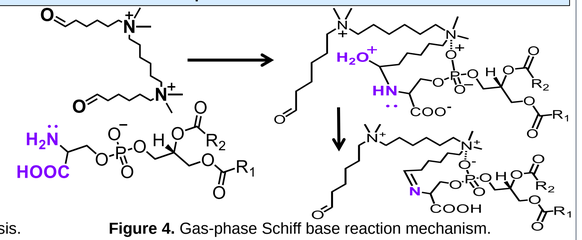

Figure 4. Gas-phase Schiff base reaction mechanism.

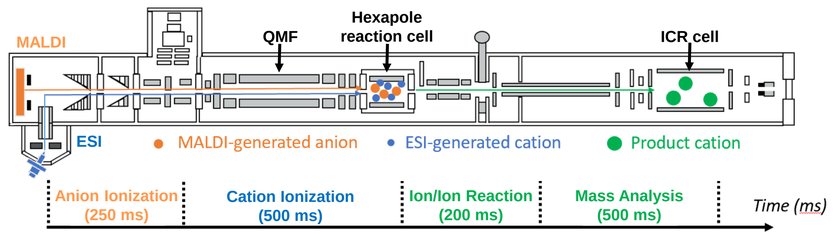

Figure 5. Ion/ion reactions are performed on a 7T solariX FT-ICR MS (Bruker Daltonics).

Gas-Phase Ion/Ion Schiff Base Formation

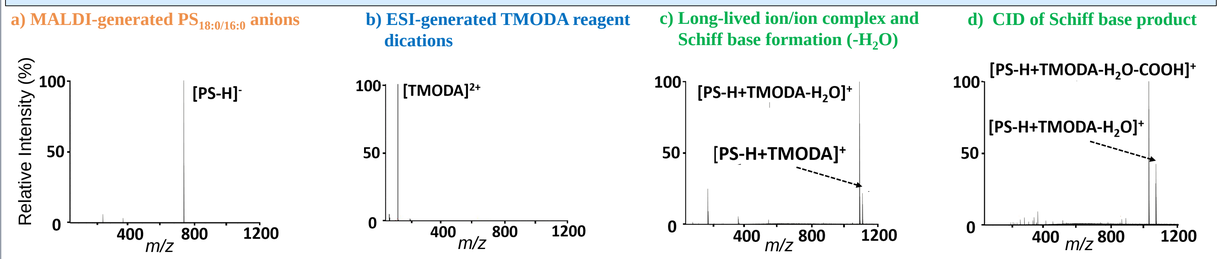

Figure 6. Charge inversion ion/ion reaction steps show: a) deprotonated $PS_{18:0/16:0}$ analyte precursor ions, b) doubly charged TMODA reagent cations, c) long-lived reaction product complex and Schiff base following ion/ion mutual storage, and d) SORI-CID of Schiff base product.

Reactive Ion/Ion Separation of Lipid **Standards:** $PE_{16:0/22:6}$(*m/z* 762.507) and $PS_{18:0/16:0}$(*m/z* 762.528)

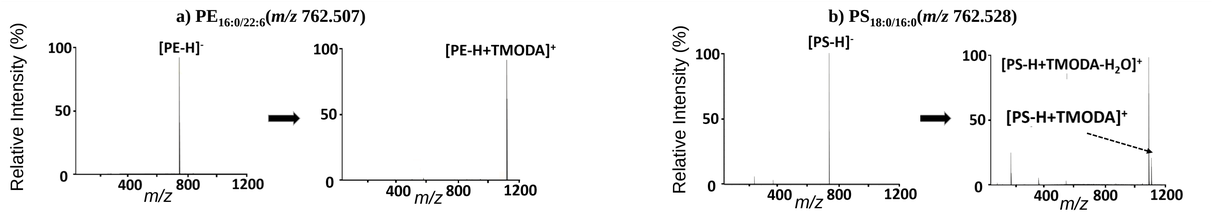

Figure 7. a) PE: a long-lived complex, but no Schiff base formation, is observed. b) PS: a long-lived complex and Schiff base formation is observed.

Separation of PE and PS Lipids from **Tissue**

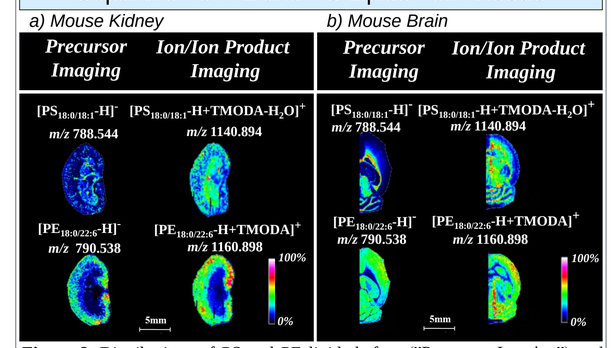

Figure 8. Distributions of PS and PE lipids before ("Precursor Imaging") and after ("Ion/Ion Product Imaging") charge inversion ion/ion reactions in mouse a) kidney and b) brain tissues.

These results confirm that spatial fidelity is maintained for PE and PS following the ion/ion reaction. Ongoing experiments are evaluating the ability of this reaction to separate truly isobaric PE and PS lipids (Δm of the precursor ions in **Figure 8** is 2 Da).

CONCLUSIONS

- The separation on phosphatidylserine from isobaric phospholipids is achieved using selective gas-phase charge inversion ion/ion Schiff base chemistry.
- The ability to separate a range of isobaric lipids in imaging mass spectrometry is currently under investigation.
- Alternative gas-phase chemistries could be used to selectively target other lipid functional groups.

REFERENCES

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