Separation of sn-Positional Isomers of Phosphatidylcholine via Differential Mobility Spectrometry

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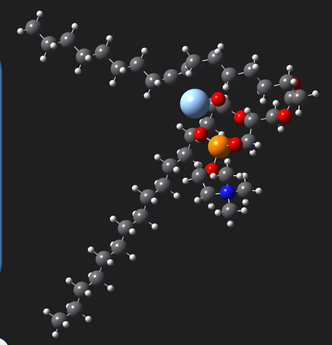
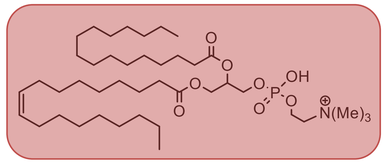


Introduction

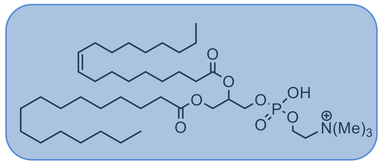
Isomeric phospholipids which vary the position of the fatty acyl chains on the glycerol backbone (sn-positional isomers) have unique physio- and biochemical properties. While current mass spectroscopic and chromatographic methods are unable to resolve such isomers, DMS has been shown to easily resolve the isomeric forms of various phosphatidylcholines (PCs) upon treatment with an Ag^+ salt.[1]

Thus, quantum mechanical calculations are employed to explore the binding motifs of the silver cation with isomeric PCs in order to justify the discrete compensation voltages (CVs) in which the isomeric forms are eluted from the DMS cell.

1-palmatoyl-2-oleoyl-sn-phosphatidylcholine (OPPC)



1-oleoyl-2-palmatoyl-sn-phosphatidylcholine (POPC)



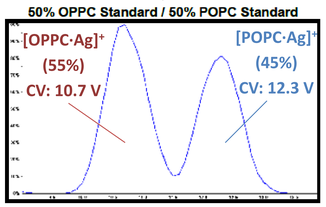


Figure 1[1]
ESI(+) of equimolar OPPC/POPC mixture with added Ag^+ salt yields two distinct peaks in the DMS ionogram.

[PC·Ag]+ in the DMS Cell

DMS Experimental Setup

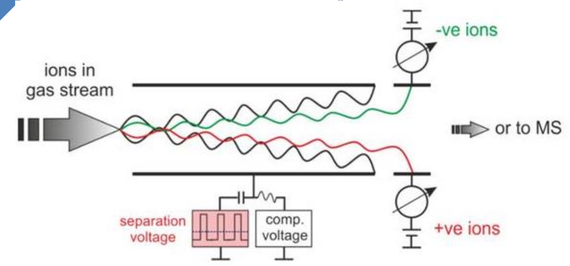
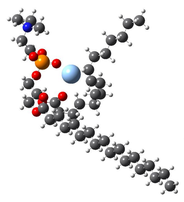


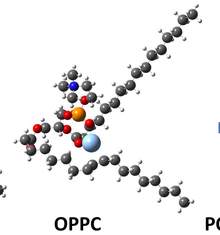
Figure 3
A typical DMS instrument[4]

A time-dependent electric field causes ions in the DMS cell to oscillate. This field is comprised of a time-dependent separation voltage (SV) and a static compensation voltage (CV). Unstable ion trajectories collide with the cell plates, while stable trajectories pass freely through the cell.

OPPC



POPC



DMS Trajectory

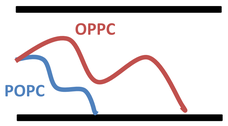


Figure 4
Binding motifs and DMS behaviour of isomeric $[PC·Ag]^+$

Differences in the partial positive charge on the Ag moiety indicate unique shielding effects on each regioisomer. Combined with the 8.5% difference in CCS and binding of the Ag^+ centre to the π-electron cloud (oleyl side-chain), this accounts for the DMS separability and unique differential mobility of both isomeric PCs.

	OPPC	POPC	% Difference
Ag Partial Charge (NBO)	0.762	0.727	4.92
Collision Cross Section (Å²)	278.11	256.36	8.48

Summary

DMS Separation Factors

- Successful separation of POPC from OPPC relies on:
  - Cationization of PC with Ag^+
  - Presence of an additional element of unsaturation in the oleyl side-chain
  - Affinity of silver to bind not only to lone pairs on heteroatoms (ie. O and N) but also to the π-electron cloud of the C=C moiety

Future Work

- Reinvestigate $[PC·Ag·X]^+$ (X= H_2O and N_2) clusters for global minimum conformation via BH to compute H_2O and N_2 binding energies

Conclusions

- Quantum mechanical calculations reinforce the DMS separability of sn-positional isomers of PCs when complexed with Ag via:
  - Varying degrees of shielding of Ag^+ centre in OPPC vs. POPC variants
  - Uniqueness of CCS between regioisomeric PCs
  - Affinity of cationic silver to the additional element of unsaturation in the oleyl acyl chain
- Overall, DMS provides a fast, alternative, and high purity separation method of regioisomeric phospholipids, compared to typical enzymatic hydrolysis methods.[5]

Computational Methods

A Basin-Hopping (BH) algorithm[2] is employed using Molecular-Mechanics (UFF) to generate low-energy candidate structures of isomeric PCs for subsequent pre-optimization at the HF/LANL2DZ level of theory.

Calculations using Density Functional Theory (DFT) employ an ONIOM based method[3] to compute cluster energetics and thermochemical parameters (see Figure 2).

Accurate cluster energies are computed through single point energy calculations at the B3LYP/6-311++g(d,p) level of theory, coupled with NBO and collision cross section (CCS) calculations to predict accurate atomic charges on the Ag^+ centre and cluster areas, respectively.

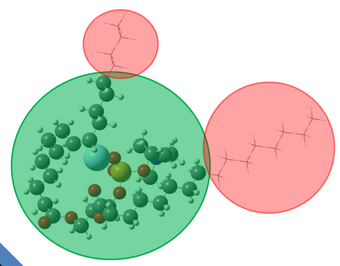


Figure 2
ONIOM layers of PC. **Green** depicts the high level of theory (**B3LYP/6-31+G(d,p)/Def2DZVP for Ag**) while **red** depicts the low level of theory (**B3LYP/3-21G**)

MM/UFF

↓

HF/LanL2DZ

↓

B3LYP/ONIOM

↓

B3LYP/6-311++G(d,p) NBO

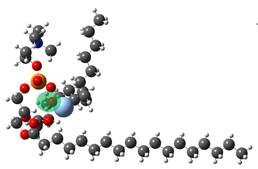
↓

Complexation with H_2O/N_2

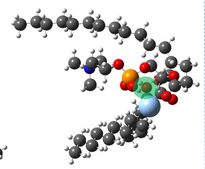
$[PC·Ag·H_2O]^+$ and $[PC·Ag·N_2]^+$

$[PC·Ag·H_2O]^+$

OPPC



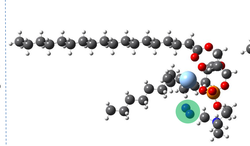
POPC



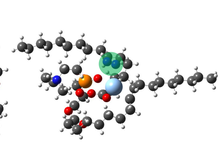
	OPPC	POPC	% Difference
Ag Partial Charge (NBO)	0.653	0.730	-11.8
Collision Cross Section (Å²)	250.45	224.63	11.5

$[PC·Ag·N_2]^+$

OPPC



POPC



	OPPC	POPC	% Difference
Ag Partial Charge (NBO)	0.674	0.613	9.91
Collision Cross Section (Å²)	266.23	226.76	17.4

Figure 5 Binding Motifs of $[PC·Ag·X]^+$ (X = H_2O/N_2)

Upon complexation with H_2O/N_2, energetic ordering of the POPC/OPPC variants reverse. Unique CCSs and Ag^+ partial charges of all H_2O/N_2 cluster remain prominent, but decrease in magnitude due to changes in the orientation of the acyl chain relative to bare $[PC·Ag]^+$.

References

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