

Mechanisms of Characteristic Phospholipid Fragmentations: a Theoretical Study

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Introduction: Phospholipids are widely distributed in cellular membranes. Phosphoglycerides typically contain two fatty acids esterified to the glycerol with a phosphate group at position 3 and are classified by the polar head group linked to the phosphate: phosphatidyl ethanolamine (GPE), choline (GPC), serine (GPS), glycerol (GPG), and inositol (GPI), all by an ester linkage, or phosphatidic acid (GPA), none. In addition, the GPG and GPI head-groups may bear additional substituents.

Low-energy CA ESI/MS/MS of the precursor anions leads to competitive losses of fatty acids and corresponding substituted ketenes and formation of fatty acid carboxylates from precursor and intermediates from ketene or acid loss. The abundance ratio of acid to ketene losses follows the order of GA (gas-phase acidity) of the polar heads groups: GPA > GPI > GPG > GPE; and MS³ of intermediates shows that the second loss tends to be opposite the first loss, forming *m/z* 153. Deuterium-labeling reveals that the acid proton of the eliminated fatty acid comes from the phosphate (GPA) and that the proton back-transferred during substituted ketene loss is from the α -carbon of the fatty acid substituent. Mechanisms based on the empirical fragmentation data were postulated, however, there are no theoretical calculations to substantiate or modify the understanding of the fragmentation processes.

Methods - Theoretical Calculations: Owing to the large size of the phosphoglyceride anions, the anion of diacetyl glycerophosphatidic (GPA) acid was selected for calculate of the potential-energy surface (PES). The molecular anion from diacetyl GPA, the smallest with α -hydrogens, is still large and flexible; hence, structural optimization of this anion and possible intermediates in fragmentation began by scanning conformational space using Monte Carlo sampling with optimization by molecular mechanics methods (MMFF). Each conformer was 'heated' to 5000 K and then annealed at 300 K and resulting distinct structures were optimized by using the PM3 semiempirical algorithm. From selected low-energy conformations, potential transition states were located by PM3; and minima and transition states were verified by vibrational-frequency analysis. At this stage, the precursor and some intermediate anions typically have over a hundred possible conformations each. Structures of *representative* minima and transition states have been optimized by using DFT to level B3LYP/6-31+G(d,p); and all minima and transition states were confirmed by vibrational frequency analyses and internal reaction coordinate investigations. Scaled thermal-energy corrections for standard conditions were applied to the results.

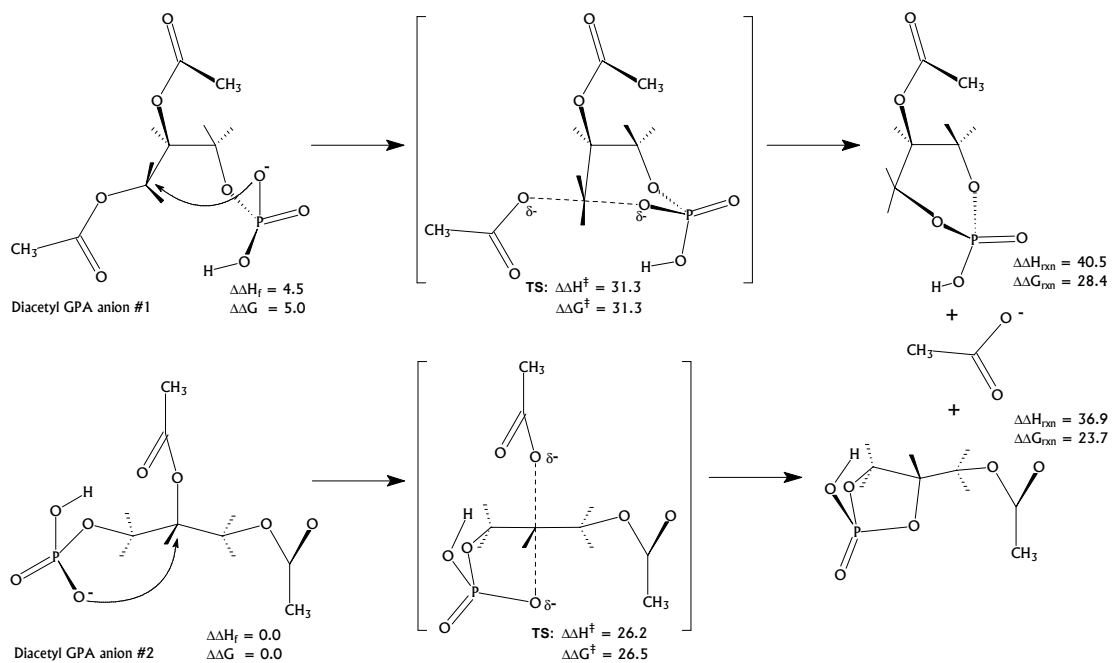
Results and Discussion - Theoretical Calculations: Initial survey calculations indicate that the loss of neutral acetic acid or ketene involves two steps rather than a single concerted step. Structural optimization at DFT level, B3LYP/6-31+G(d,p), suggest the following mechanisms and energetic requirements:

- Acetate is displaced by an internal SN₂ displacement attack by one of the phosphate oxygens upon the C1 or C2 carbon of the glycerol backbone, whether in the precursor or intermediate ions (Scheme 1). Geometric constraints do not allow formation of the acetate close enough to the acidic proton on the phosphate group for proton transfer, thus acid loss is by some other mechanism. Average relative reaction free energy and barrier height are: $\Delta\Delta G_{rxn} = \sim 23$ kcal/mol and $\Delta\Delta G^\ddagger = \sim 25$ kcal/mol.
- Loss of ketene results from transfer of a carboxylate moiety from backbone to phosphate oxygen followed by abstraction of an α -carbon proton by the resulting alkoxide ion (Scheme 2). The phosphate anion is too weak a base to abstract an α -carbon proton and form a necessary stable intermediate. The proposed pathway is thus consistent with D-labeling experiments. The overall reaction free energy is: $\Delta\Delta G_{rxn} = 12.5$ kcal/mol, and the maximum barrier is: $\Delta\Delta G^\ddagger = 35$ kcal/mol.
- Loss of acetic acid by a charge-remote pericyclic mechanism is feasible and may be observed by high-energy CA MS/MS. This mechanism has been observed in more basic phosphoglyceride anions but not GPA under low-energy CA. Average relative reaction free energy and barrier height are: $\Delta\Delta G_{rxn} = \sim 10$ kcal/mol and $\Delta\Delta G^\ddagger = \sim 35$ kcal/mol.
- Mechanism for loss of acetic acid that would be competitive with ketene loss has yet to be defined, although a high-energy route involving a penta-coordinate phosphorous has been discovered.

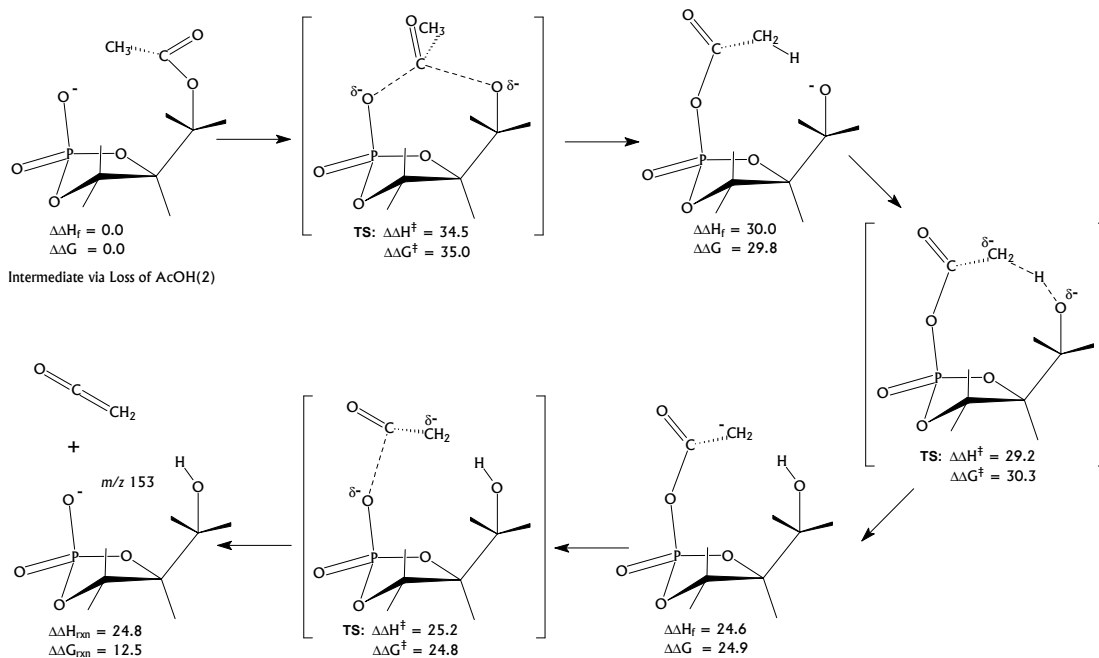
This project is a work under progress. Mechanistic refinement and calculations to higher level are needed for better values of barrier energetics of transition states and minima to characterize the PES.

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Scheme 1: Proposed Mechanism - Loss of Acetate from Diacetyl GPA anions.



Scheme 2: Proposed Mechanism - Loss of Ketene from Intermediate formed by AcOH loss.



Relative enthalpies and free energies are in kcal/mol (TS = Transition State).