Demonstrating lyso-PAF Acetyltransferase Activity In Sea Urchin Embryo

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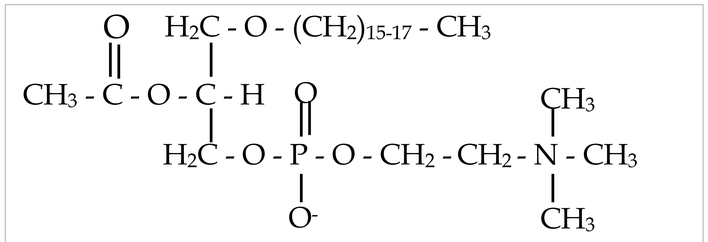
Introduction

- Platelet-activating factor (alkylacetylglycerolphosphocholine; PAF) is a potent signaling phospholipid which has been found in numerous cell types in every physiological system studied to date. In reproduction, PAF is found to have a variety of roles, for example: in ovulation, sperm function, and early preimplantation development.



1 - O - alkyl - 2 - acetyl - sn - glycero - 3 - phosphocholine

Figure 1: Structure of PAF

Note: Jiu-ming, Zhu, Joe, Massey, William, & Roudebush (2005). Physiological Roles of Platelet-activating Factor in Mammalian and Human Reproduction.

Mechanism of PAF

- PAF is produced by two distinct pathways

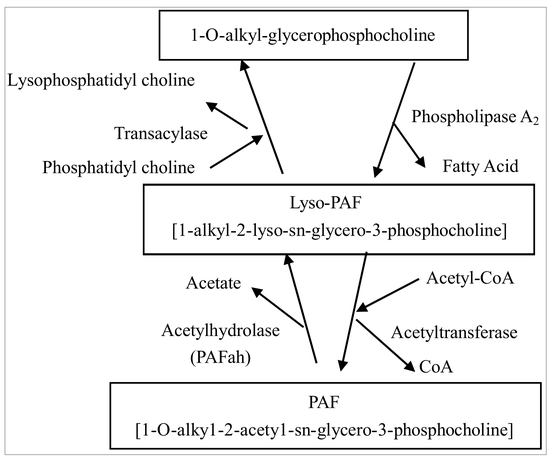


Figure 2: Mechanism of PAF

Note: Jiu-ming, Zhu, Joe, Massey, William, & Roudebush (2005). Physiological Roles of Platelet-activating Factor in Mammalian and Human Reproduction.

Do Sea Urchin Embryo Contain Acetyltransferase?

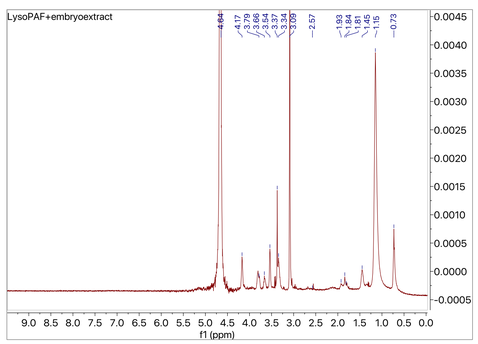
- Individual Lytechinus variegatus sea urchins were injected with 0.5 mL KCl (0.5M) to induce gamete shedding and pooled accordingly.
- Oocytes were inseminated with spermatozoa in synthetic sea water and cultured (for approximately 90 minutes) to produce two-cell stage embryos.
- The two-cell stage embryos were selected and cultured in synthetic sea water for 24-hours at 22ºC.
- Embryos were then frozen and thawed to induce cellular lysis.
- Lyso-PAF was dissolved in deuterated water.
- Lyso-PAF alone, Lyso-PAF with XuM Acetyl-CoA, Lyso-PAF with XuM AcetylCoA and embryo extract, and Lyso-PAF with embryo extracts were all assessed with H1 NMR and C13 NMR after a 30-minute incubation at room temperature.
- These spectra were obtained via a 500mHz NMR.

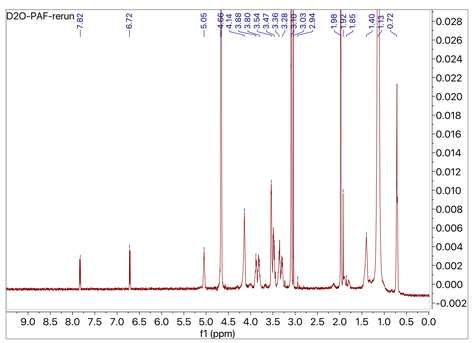
Future Work

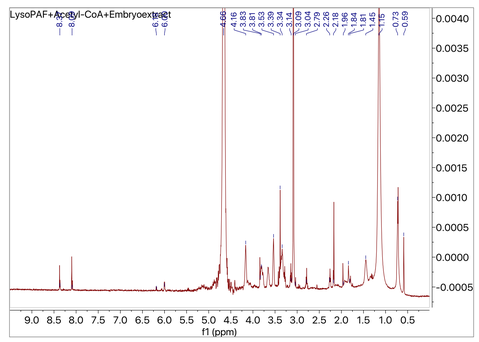
- Future directions for this research will include bioinformatics analysis of the annotated sea urchin genome to try and identify the acetyltransferase gene that could be responsible for Lyso-PAF acetylation.

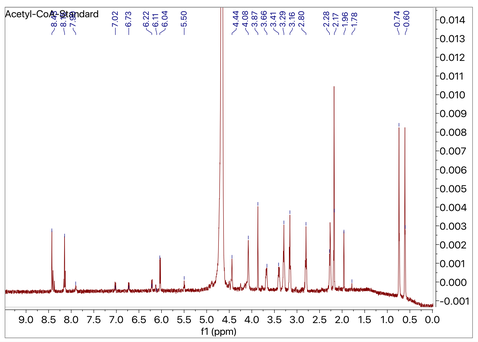
Results

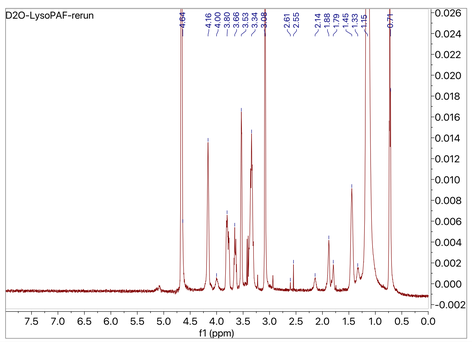
- Initial results revealed that the acetyl-CoA peak on the NMR resides in the same location as where we expect the acetylated Lyso-PAF peak to reside. Therefore, additional experiments are planned to try and resolve these two peaks.

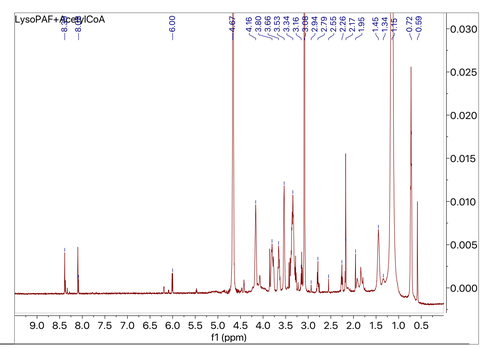












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