Phospholipid Vesicles as Microreactor Milieu for Biochemical Activity of FadD10

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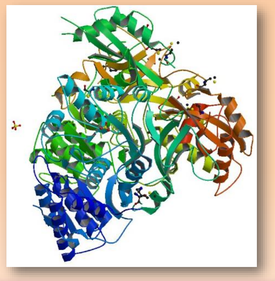
INTRODUCTION

Lipid-bound vesicles are thought to be the precursors to modern day cells – however, for vesicles to act as cells, they need to be able to include proteins within them. In this study, FadD10 protein from *Mycobacterium tuberculosis* was selected to test the viability and function of a protein inside a vesicle. Vesicles encapsulating the FadD10 enzyme were prepared using the inverse emulsion method, and the necessary substrates (a long-chain saturated fatty acid and ATP) and cofactor (Mg^{2+}) were included in the solution. Texas Red-DHPE was used as a membrane staining fluorescent dye to observe the changes in morphology, appearance, and structure of the vesicles.

FadD10 Protein Structure

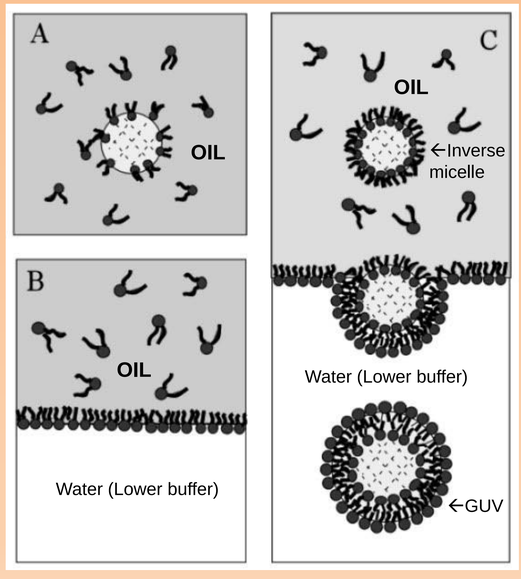


INVERSE EMULSION TECHNIQUE

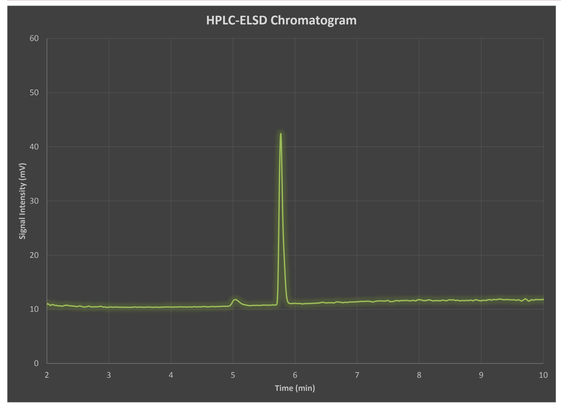
A. Water droplets (reaction mixture) are emulsified in mineral oil containing phospholipids (DOPC) and placed on top of *lower buffer*. The hydrophilic head groups face the water droplet and hydrocarbon chains face the oil forming **inverse micelles**.

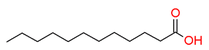
B. The droplets are centrifuged through the oil-water interface.

C. In this process, the inner and outer leaflets of the bilayer meet to form a Giant Unilamellar Vesicle (GUV)

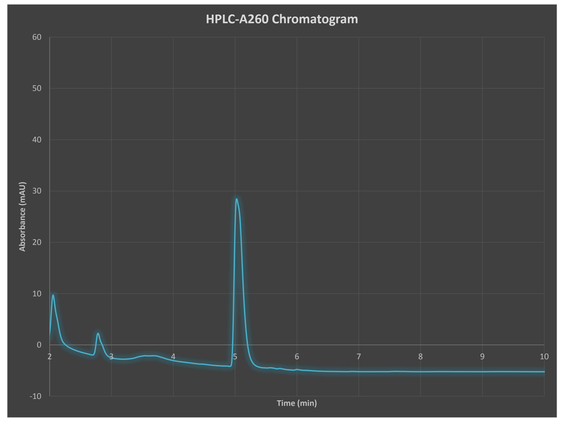


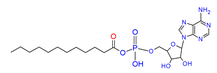
HPLC





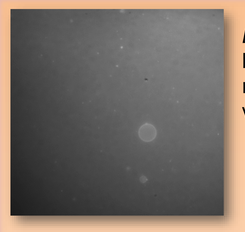
The peak at t = 5.77 min corresponds to Dodecanoic acid (the long-chain fatty acid), shown above.





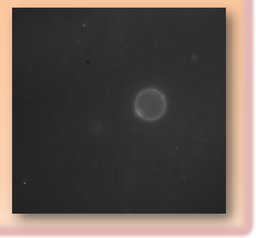
The peak at t = 5.03 min corresponds to Dodecanoyl-AMP, a high-energy intermediate produced by the FadD10 enzyme.

CONTROL ANALYSIS



Left: Vesicles formed without adding ATP in the upper buffer, thus disabling the function of the enzyme. The membranes were stained with Texas Red-DHPE and visualized under Texas Red channel.

Right: Vesicles formed without the external addition of fatty acid, thus omitting the principal substrate of the enzyme.



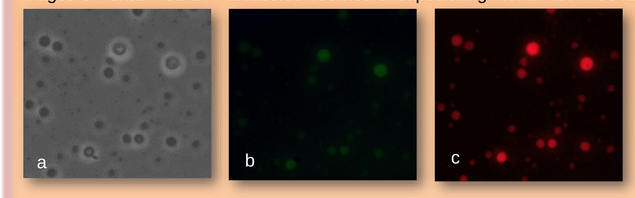
REFERENCES

1) Introduction of Unilamellar Vesicles Using an Inverted Emulsion Sophie Pautot, Barbara J. Frisken, and D. A. Weitz, *J Biol Chem*. **2013 Jun 21**; 288(25):18473-83.

2) Structures of Mycobacterium tuberculosis FadD10 protein reveal a new type of adenylate-forming enzyme. Liu Z1, *Loerger TR*, Wang F, Sacchettini JC, Langmuir **2003**, 19, 2870-2879

VESICLE FORMATION

Images of Texas Red-DHPE labeled vesicles encapsulating Alexa Fluor 488 labeled FadD10

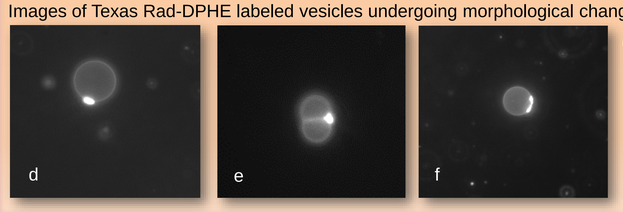


a – bright-field with phase contrast

b – Alexa-Fluor 488 channel

c – Texas Red channel

Images of Texas Rad-DPHE labeled vesicles undergoing morphological changes



d, e, f – Texas Red channel

ANALYSIS

As shown by the images, there was a bright spot present in the vesicles prepared with the substrates that was not present in the controls. The bright spots are where the fluorescent dye binds to the products of the reaction catalyzed by the FadD10 protein. This reveals that the FadD10 enzyme was functional inside the vesicle, as it could carry out the reaction and produce the expected products. As mentioned before, this is further confirmed by the lack of these bright areas in the controls, eliminating the possibility that they could be due to factors other than the enzyme. In addition, this also shows that the vesicle membrane is permeable to the substrates.

CONCLUSIONS

We demonstrated the encapsulation of a protein inside a vesicle and tested its' functionality. The protein produced the products successfully, showing that proteins can conduct their normal function inside a vesicle. It also showed that the substrates of the FadD10 enzyme could pass through the vesicle membrane.

FUTURE RESEARCH

The goal of the Devaraj lab is to create artificial cells from the bottom up, starting with vesicles and adding the various features that characterize cells to them. Encapsulating proteins inside a vesicle is one part of this larger goal. A future step would be to incorporate important proteins into vesicles because it has been proven that they function properly.