Elizabeth Holt (Chemistry '18) and Mathew Hurley, Ph.D.: Protein Functionalized Catanionic Vesicles

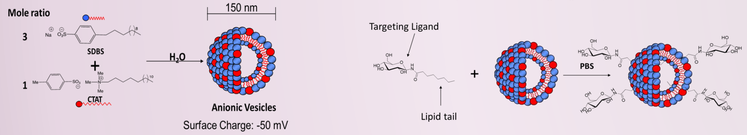
Abstract

Hydrophobically modified carbohydrates were incorporated into CTAT/SDBS vesicles and the carbohydrate presence was quantified via sulfuric acid assay. Two different glucose conjugates were used to test the functionalization of vesicles. The first one, n-octyl-β-D-glucoside did not incorporate into the vesicles. The second conjugate n-dodecyl-β-D-glucopyranoside had a 41.3% incorporation into the vesicles with a concentration of 26.1 µM.
Hydrophobically modified glucose reacted with acrolyl chloride in order to produce an acrylic ester. A TLC of this reaction mixture showed two major spots. TLC mass spec analysis shows peaks at 369.9 m/z and 424.0 m/z representative of the product.
A TLC mass spec analysis of the cysteine conjugation showed a peak at 470.1 m/z, which is only 0.2% off from the expected peak for the monocysteine conjugate. The monocysteine conjugate was incorporated into bare vesicles with a concentration of 73.4 µM.

Background

Vesicles are formed spontaneously when two surfactants having oppositely charged polar heads are mixed in the correct ratio. These vesicles are soft and spherical. In this experiment vesicles were prepared with CTAT and SDBS in PBS. These particular vesicles form a lipid bilayer and have an interior lumen. They will be either cationic or anionic depending on which surfactant is used in excess. The anionic vesicles are about 150 nm in diameter and cationic vesicles are roughly 250 nm in diameter.

Vesicles are easy to functionalize by attaching molecules with a polar head and long hydrophobic tail, by anchoring the tail into the lipid bilayer. By doing this carbohydrates can be attached and built upon to attack larger proteins and peptides. Hydrophobically modified carbohydrates can be incorporated into the lipid bilayer of the vesicles. Their presence can be determined via lectin binding experiments (park et al.). These vesicle systems have potential for being vaccine platforms and drug delivery devices. This research mostly targets the use for vaccine platforms.



Thomas et al. JACS **2009**, 131, 5471-5477 Park et al. Soft Matter **2008**, 4, 1916-1921

Other uses for these vesicles include the separation of charged organic solutes, vaccine and drug delivery, colloid and interface science.

Method

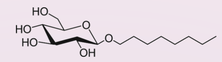
Mono and diester conjugates were made in a two step synthesis. In the first step octyl glucose and DMAP were dissolved in DCM and reacted with triethylamine and acrolyl chloride. The mixture produced in this step was then reacted with L-cysteine HCL and TCEP HCL, after being dissolved in PBS. NaOH was added to increase the pH to 7. The mixture from step one and step two were analyzed with TLC MS to verify the identity of the reactants.

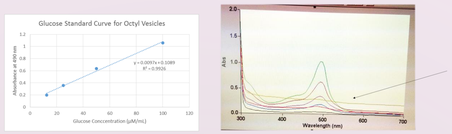
Vesicles were formed by mixing SDBS and CTAT and the reaction mixture with the monoester in PBS and stirring overnight until vesicles were formed. Vesicles were purified using s size exclusion column in increments on 1.0 mL. An assay using phenol and sulfuric acid was performed on the vesicle reactions in order to determine concentration of carbohydrates present in each fraction.

References

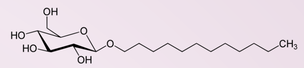
Kaler et al. Science **1989**, 245, 1371-1374
Oppolzer et al. Helvetica Chimica Atca **1981,** 64, 2802-2807
Park et al. Soft Matter **2008**, 4, 1916-1921
Lin et al. J. Am. Chem. Soc, **2010**, 132, 16805-16811

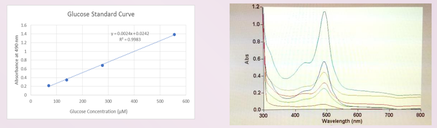
Results



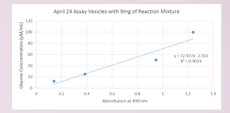


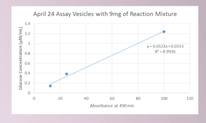
	Concentration	Absorbance
Bare Vesicles	-5.4 µg/mL	0.057 nm
C-8 Vesicles	-1.9 µg/mL	0.09 nm





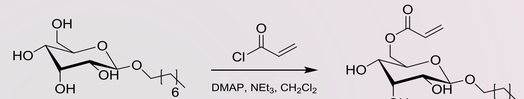
	Concentration	Absorbance
C-12 Vesicles	103.3 µg/mL	0.2721 nm



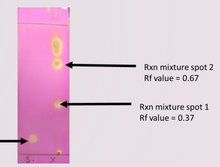


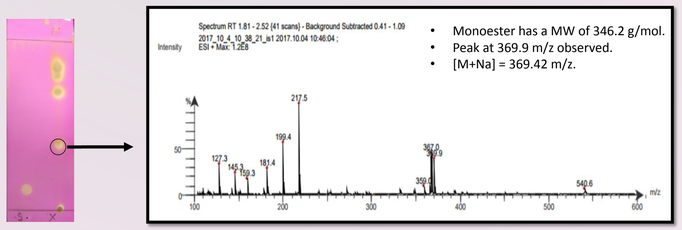
	Concentration	Absorbance
9mg Rxn Vesicles	65.0 µg/mL	0.92628 nm
9 mg Rxn Vesicles	73.4 µg/mL	0.92628 nm

First Step

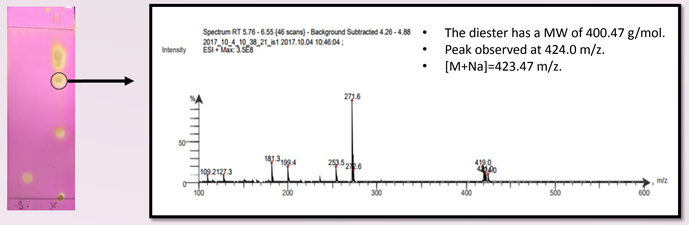


Synthetic Scheme: Step 1
Hydrophobically modified glucose reacted with acrolyl chloride in order to produce an acrylic ester.



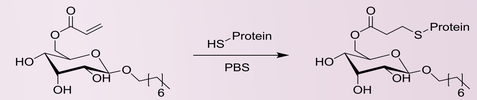


- Monoester has a MW of 346.2 g/mol.
- Peak at 369.9 m/z observed.
- [M+Na] = 369.42 m/z.



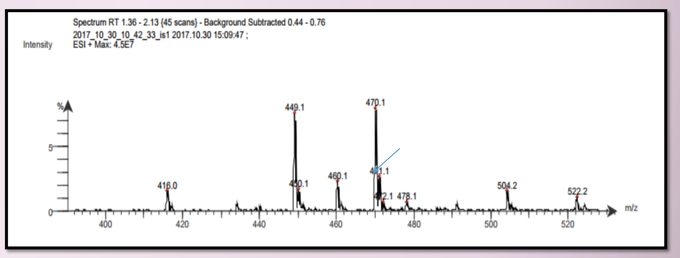
- The diester has a MW of 400.47 g/mol.
- Peak observed at 424.0 m/z.
- [M+Na]=423.47 m/z.

Second Step



Synthetic Scheme: Step 2
Our alpha/beta unsaturated ester is then reacted with the thiol group on cysteine to attach a protein to one of the alcohol groups on the modified glucose

Reaction Mixture



- Peak observed at 470.1 m/z
- MW of monocysteine conjugate 467.6 g/mol.
- In positive mode we expect to see a peak at 468.6 m/z
- Off by 1.5 mass charge units or 0.2%
- Peak at 449.1 m/z can be attributed to the loss of a carboxylic acid and addition of a sodium atom.

Conclusion

The dodecyl glucose conjugate was successfully incorporated into the SDBS/CTAT vesicles. This along with the desired monocysteine conjugate show promising results. The next step is to incorporate the monocysteine conjugate with the vesicles. This will then allow for further functionalization and may help with creating vaccine platforms that mimic the body's cells with targeting agents that only attack specific targets. The monocysteine conjugation showed good incorporation into glucose vesicles with a concentration of 73.4 µM.