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Using Lipid Nanodiscs for the *in vitro* Glycosylation of Membrane Proteins

Rachel F. Dolan and Gabriel A. Cook

Department of Chemistry, Oklahoma State University, Stillwater, OK 74078

Abstract

Membrane proteins are involved in a number of human diseases are important to study. Due to the fact that these proteins are, by nature, insoluble in aqueous solutions, they require detergent or lipid environments that resemble cell membranes to remain soluble. Nanodiscs can be used to prevent these proteins from precipitating. Nanodiscs are patches of phospholipid bilayer that is encircled by membrane scaffold protein and mimic the thickness of the human cell membrane. This allows for membrane proteins to be studied in a native-like environment. In our lab we study a specific group of proteins that are post-translationally modified. These proteins are glycoproteins which have sugar groups attached to their sidechains. It is also important to understand how proteins change when a sugar is attached to them. This will be examined by first inserting the membrane protein into nanodiscs and then attempting to glycosylate them using in vitro glycosylation with the enzyme N-Glycosyltransferase (NGT). We will show that membrane proteins that are inserted into nanodiscs can be glycosylated using these methods.

Introduction

Proteins that are found in the lipid bilayers of cells, also known as membrane proteins, are involved in certain human diseases, such as muscular dystrophy. These types of human diseases can be a big problem in the United States and the world. The main reason for this is that there is no cure for muscular dystrophy, only treatments for the symptoms. In order to advance research for these diseases, it is crucial to be able to look at how the membrane proteins interact within the human body.

The structure of the membrane protein needs to be studied in order to determine how it works in the human body. A process called nuclear magnetic resonance spectroscopy (NMR) is typically used to do this. The problem with certain membrane proteins, such as those that are involved in muscular dystrophy, is that the structure of the protein breaks up when submerged in the NMR solution. This is a problem because the denatured protein's structure is broken up and the structure cannot be determined. Nanodiscs are effective at incorporating membrane proteins to be visualized using NMR. Nanodiscs are patches of phospholipid bilayer that is encircled by membrane scaffold protein, which controls the size of the nanodisc. Nanodiscs mimic the thickness of the cell membrane, which allows the membrane proteins to feel as though they are in their native environment.

Nanodiscs can also be effective for the in vitro glycosylation of membrane proteins. It is known that membrane proteins can be glycosylated using micelles, but it is unknown whether the same can be done in nanodiscs. Nanodiscs can be ideal for glycosylation because it allows access to both sites of the membrane protein. Glycosylation will allow the visualization of how sugars affect membrane proteins, whether it be a change in structure or dynamics. It is predicted that the membrane proteins will successfully be glycosylated in vitro using nanodiscs.

Materials and Equipment

- Kanamycin (antibiotic)
- Bacterial Cell Stock with plasmid for MSP expression
- Ultraviolet-visible (UV) spectrophotometer
- Centrifuge
- Sonicator
- Nickel Column and various buffers
- Lyophilizer

Figures

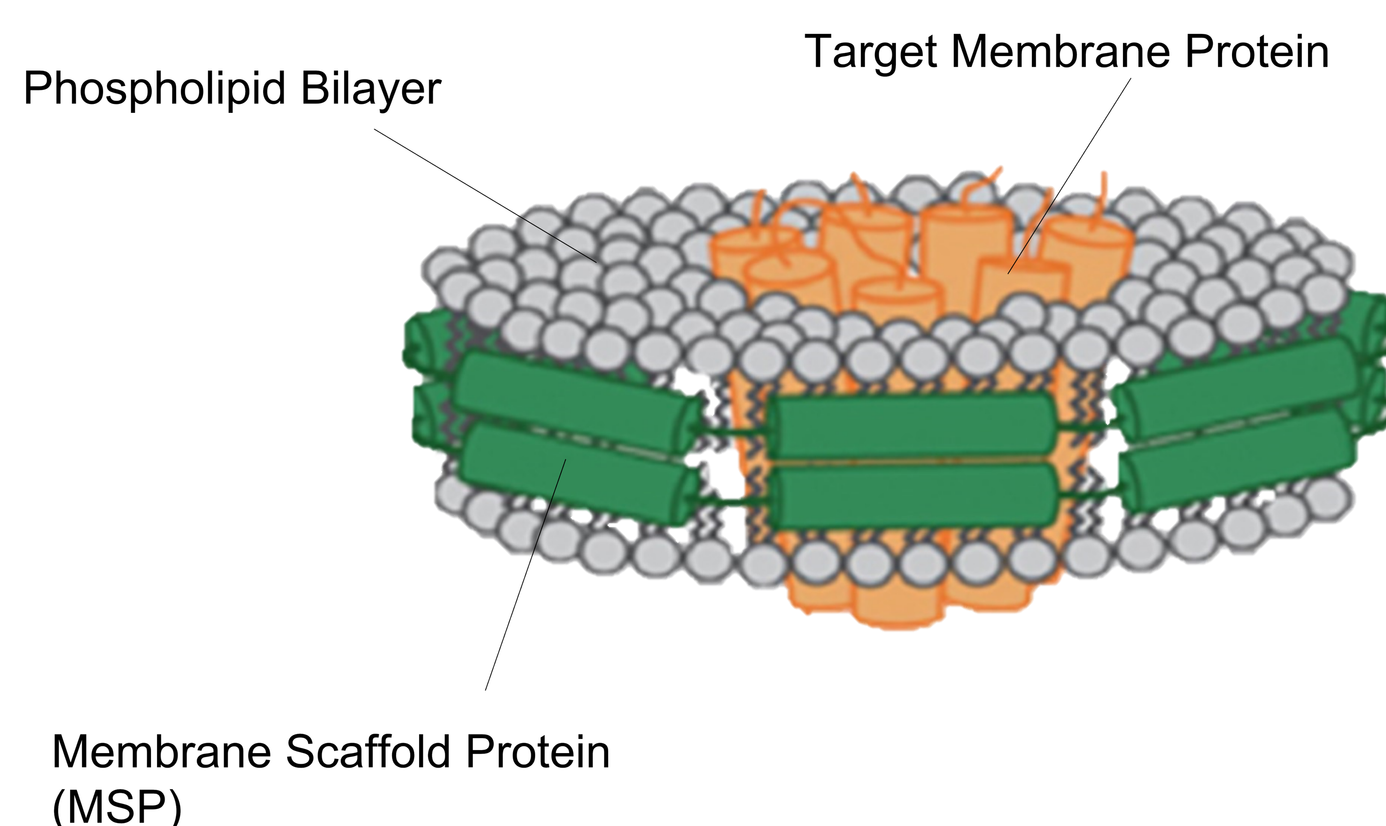

Figure 1. Structure of the nanodisc

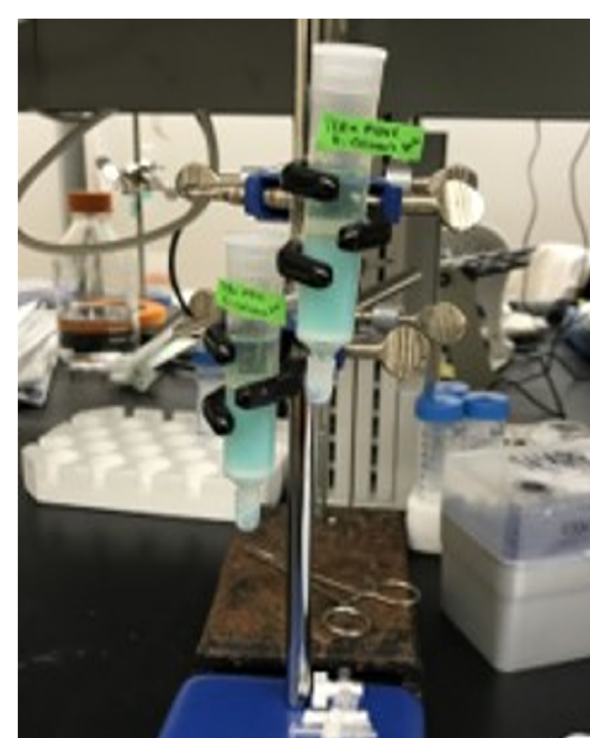

Figure 2. Purifcation with nickel affinity chromatography

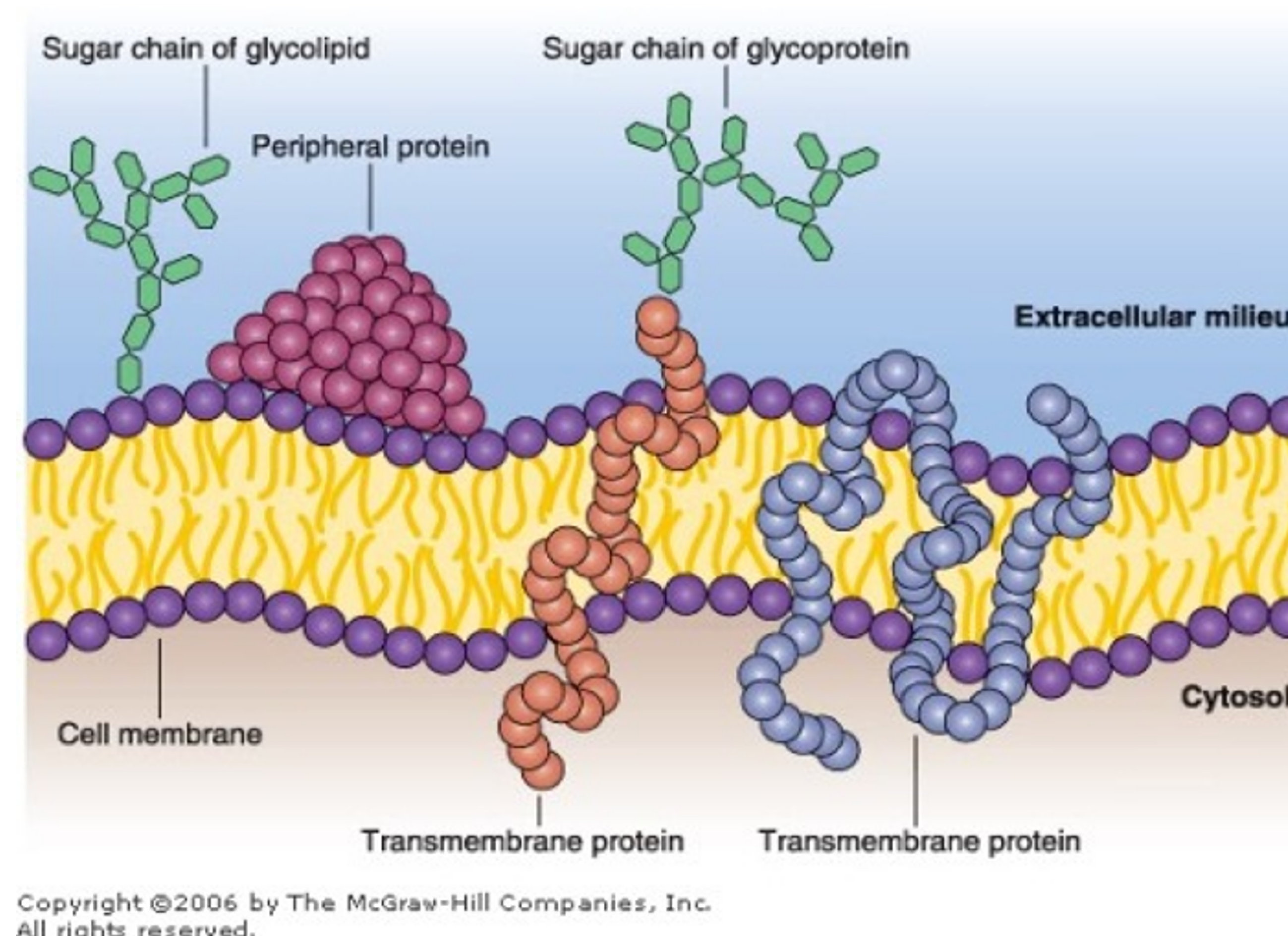

Figure 3. Visual of cell membrane and proteins with sugars attached.

Methods

Expression:

1. Protein is grown using kanamycin, Luria-Bertani (LB) broth, kanamycin, a cell starter containing LB, kanamycin, and a cell stock with plasmid for MSP expression.
2. The growth is test in a UV spectrometer at 600 nm to check the cell count.
3. The cell growth is then induced with isopropylthio- β-galactoside (IPTG) to promote bacteria activity.
4. Cell growth is tested again using the UV spectrometer, and the cell growth is then centrifuged at 6500 rpm for 25 minutes at 4 °C. The cell pellet is saved and stored in a -80 °C freezer

Purification and Dialysis:

1. Cell pellets are lysed using phosphate buffer and then centrifuged. This time, the supernatant is saved and stored at 4 °C.
2. The supernatant is ran through a nickel column using a variety of washes, and about 7 mL are collected.
3. The collected sample is dialyzed for 24 hours in a buffer bath that is changed once.
4. The sample is then concentrated and can be used to form nanodiscs.

Glycosylation:

1. Tobacco etch virus (TEV) and dithiothreitol (DTT) is added to MSP sample and is dialyzed in cold room overnight.
2. Sample is ran through nickel column and dialyzed.
3. Nanodiscs are prepped with γ-sarcoglycan and dialyzed in 20mM phosphate buffer. Sample is concentrated using centrifugence.
4. Samples are separated into two separate tubes, and UDP-glucose is added to the reaction tube. Samples are left on rotator for 24 hours.
5. Mixtures are dialyzed separately, frozen, then put on lyophilizer to dry.

Results

The membrane scaffold protein was successfully purified and was able to spontaneously form into nanodiscs when combined with the lipids and target membrane proteins. Results from mass spectrometry are still pending to determine if glycosylation was successful.

Future Directions

After successfully glycosylating membrane proteins in vitro using small sugars, future goals include attaching larger sugars that are more representative of sugars in the body. The goal is to determine how sugars modify membrane proteins, whether it be through a change in structure or dynamics of the protein. This could hopefully lead to a better understanding of how these proteins cause or are involved in muscular dystrophy.

References

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