On Chip Production of GM1 for the Treatment of Huntington's Disease

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Abstract:

Huntington's Disease (HD) is a neurodegenerative disorder that causes physical, behavioral, and cognitive deterioration. This disease stems from a mutation in the Huntington protein that depletes levels of an essential glycolipid, ganglioside GM1. While there is currently no cure for HD, mouse model studies have shown that restoring levels of GM1 can slow and possibly halt symptoms of HD. However, GM1 is challenging to synthesize and costly to extract from brain tissue. Therefore, this study seeks to produce GM1 *ex vivo* using a microfluidic device. To advance this goal, the biosynthetic pathway that converts the ganglioside GM3 to ganglioside GM1 was mimicked in a single pot reaction, and a method that quickly identifies the reaction products was developed. The primary identification technique used was Thin Layer Chromotography (TLC). The initial lab protocol was optimized to give results with more distinct separation. Total internal reflection fluorescence microscopy (TIRFM) was also used to characterize the reaction products. This method leveraged the interactions between azide-linked sugars and a cycloalkyne fluorophore, dibenzocylcooctyne (DBCO) to fluorescently label and detect GM1 products.

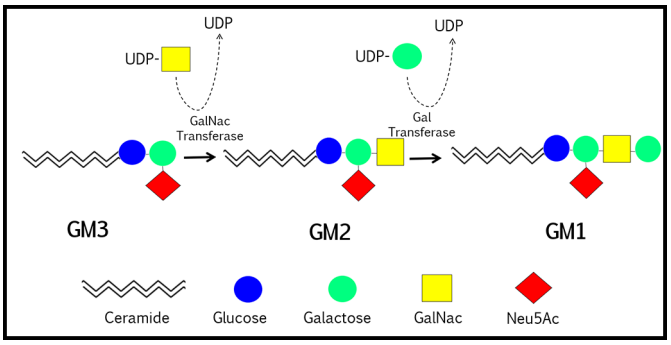

Figure 1: Biosynthetic pathway to ganglioside GM1 from ganglioside GM3.

Summary of Research:

GM1 is a promising therapeutic for HD. However, difficult synthesis and expensive extraction from native tissue have prevented clinical application of GM1. This project seeks to simplify the preparation of GM1- by mimicking its biosynthesis from ganglioside GM3, in a microfluidic device. During this biosynthetic pathway, GM3 undergoes two glycosylation reactions to produce GM1 (Figure 1). The first stages of this project aimed to replicate the GM3-to-GM1 reaction sequence using a single-pot approach. This required the development of a quick and reliable way to determine the success of the reaction before the project could proceed.

The first technique used to characterize the products of the GM3-to-GM1 reaction was thin layer chromatography (TLC). The goal was to design a protocol that would consistently and distinctively separate GM3, GM1, and GM2 intermediates. In the initial TLC protocol, samples were suspended in methanol and the TLC plate was developed in a running solvent with chloroform/methanol/0.2% aqueous calcium chloride (55:45:10, v/v/v) (Figure 2A). This protocol failed to separate the reaction products, and it was altered by varying ratio of the running solvent. During TLC trials, samples were either suspended in methanol or in a 50/50 mixture of chloroform and methanol. Pure, commercial samples of GM3 and GM1 were used to test the altered TLC protocol (Figure 2). Protocols that provided clear separation were then used to analyze the products of the GM3-to-GM1 single-pot reaction.

The second method used to characterize the reaction products was total internal reflection fluorescence microscopy (TIRFM). TIRFM can selectively illuminate and excite fluorophores at the surface of a supported lipid bilayer (SLB), and thus, could be used to confirm the presence of fluorescently labeled GM1 in a SLB. To do so, GM3 lipids were placed into an SLB and underwent reactions with normal GalNac sugars and special galactose (gal) sugars carrying azide groups.

After the reaction, the azido-gal sugars that attached to glycolipids could couple with the cycloalkyne fluorophore, DBCO, to fluorescently label ganglioside products in the SLB. Since the gal sugar is the second sugar addition (Figure 1), fluorescent tags should only attach to GM1 products. The fluorescent signals in the SLBs were measured under the TIRF microscope and analyzed with ImageJ software [2].

Results and Conclusions:

The best TLC protocol use a running solvent with chloroform/methanol/0.22% aqueous calcium chloride (60:40:10, v/v/v). The samples were suspended in 50/50 chloroform and methanol. This protocol showed clear separation between GM1, GM2 intermediates, and GM3 (Figure 3). The average fluorescence intensity measured for each sample is reported in Figure 4. The results show that the target reaction in it, had the highest fluorescence intensity whereas the various controls had significantly lower intensities. This suggests that some GM1 was produced in the reaction and that its production can be detected by this method.

Future Work:

The improved TLC protocol and the TIRF experiments will be used to rapidly analyze the products of the GM3-to-GM1 reactions performed in the next phase of the project. Additionally, other methods such as high-performance liquid chromatography (HPLC) and mass spectrometry can be considered for further identifying products.

Acknowledgements:

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References:

[1] Di Pardo A, et al. Proc. Natl Acad. Sci. U.S.A. 2012, 109, 3528.3533.

[2] Schneider, C. A.; Rasband, W. S., and Eliceiri, K. W. Nat. Meth. 2012, 9, 671.675.

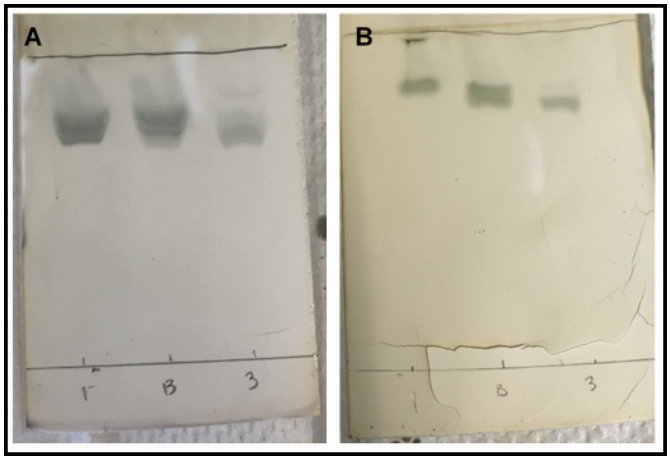

Figure 2: Early TLC plate tests. Figure 2A shows the initial TLC protocol. Figure 2B shows an improved protocol using a running solvent with chloroform/methanol/0.2% aqueous calcium chloride (60:40:10, v/v/v). All samples were suspended in methanol. For each plate, GM1 is spotted on the left, GM3 is spotted on the right, and there is a spot with both in the middle.

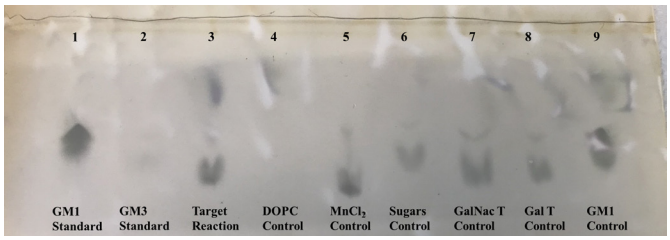

Figure 3: A TLC plate with the improved protocol. The top marks are likely other sugars or impurities. Columns 1 and 9 show GM1. Column 5 roughly indicates where GM3 is located. The remaining columns are GM2 intermediates.

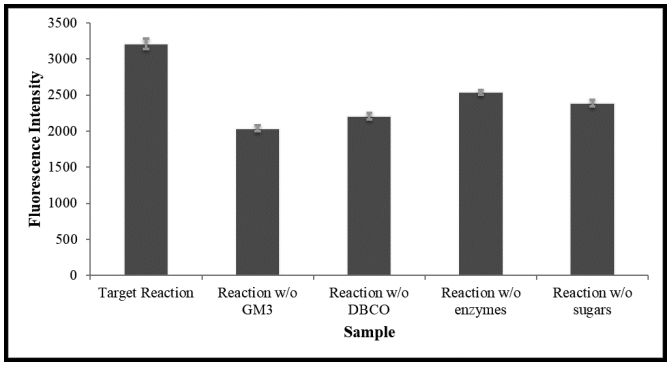

Figure 4: A comparison of the average fluorescence intensities of the SLB samples from the TIRF experiment. The first column has the target reaction. The other columns are controls used to determine the amount of background fluorescence or non-specific binding of DBCO.