Developing an enzyme assay to measure glucosylceramide synthase activity in the presence of HIV-1 gp-120

Kory M. F. Joe and Lisa Smith

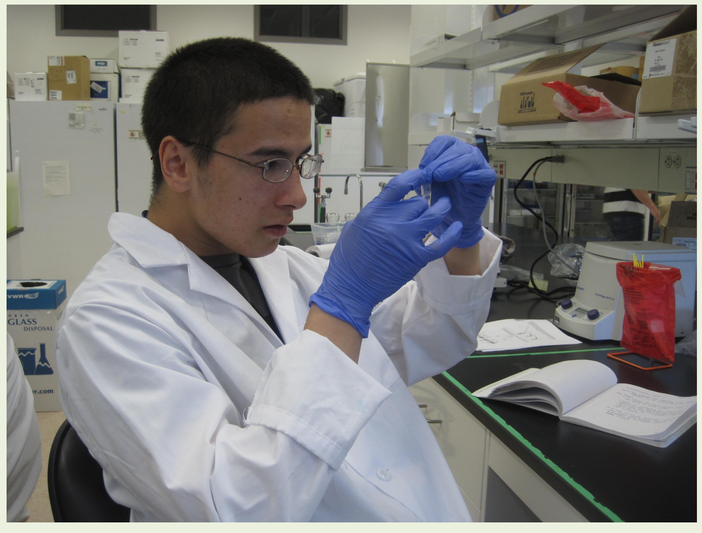
Abstract

Human immunodeficiency virus-1 (HIV-1) glycoprotein-120 (gp120) is known to targets lipid rafts for entry into the host organism's cells (S, Fuchs, & Schiller, 2011). HIV-1 gp120 has been noticed to also cause elevated levels of ceramide (a precursor molecule for glycosphingolipids) within cells (Campbell, Crowe, Mak, 2001). Patients with neurological defects, such as Alzheimer's disease and HIV associated dementia (HAD), are known to have elevated levels of ceramide within their cells (Uchida et al., 2002). I hypothesize that the activity of glucosylceramide synthase (GCS), an enzyme that bonds a glucose molecule to ceramide, converting it to glucosylceramide, might be negatively affected by HIV-1 gp120. To test whether GCS is functioning normally, I am developing an enzyme assay to test enzyme activity in the presence of HIV-1 gp120.

Background

Infections and diseases are caused by various pathogens that target host cells for replication. Some pathogens target host cell lipid rafts for cell entry. Lipid rafts are plasma membrane microdomains composed of high concentrations of glycosphingolipids and cholesterol (Vieira et al., 2010). Human immunodeficiency virus (HIV) is a pathogen known to targets lipid rafts to infect target cells using its envelope glycoprotein, gp120 (Verma, 2009). HIV-associated dementia (HAD) is a complication seen in some patients with HIV infection. HAD is a neurocognitive disorder that causes cognitive impairment (Uchida et al., 2002). Elevated levels of ceramide have been seen in patients with HAD (Verma, 2009). Ceramide is a waxy lipid molecule that is the precursor molecule for glycosphingolipids. Too much ceramide is toxic for the cell, because a high concentration of ceramide can cause apoptosis. Glucosylceramide synthase (GCS) prevents the negative effects of too much ceramide by converting ceramide into the non-toxic glucosylceramide (Uchida et al., 2002).

I worked to establish an enzyme assay to measure GCS activity within a cell in the presence of HIV-1 gp120. Using this enzyme assay, further studies will investigate if gp120 affects GCS activity resulting in the observed elevated levels of ceramide. This enzyme assay uses thin layer chromatography (TLC) to determine whether or not ceramide is converted to glucosylceramide in the presence of HIV-1 gp120.



Kory M. F. Joe working on lab preparation.

Figure 1. Flow chart of proposed methods

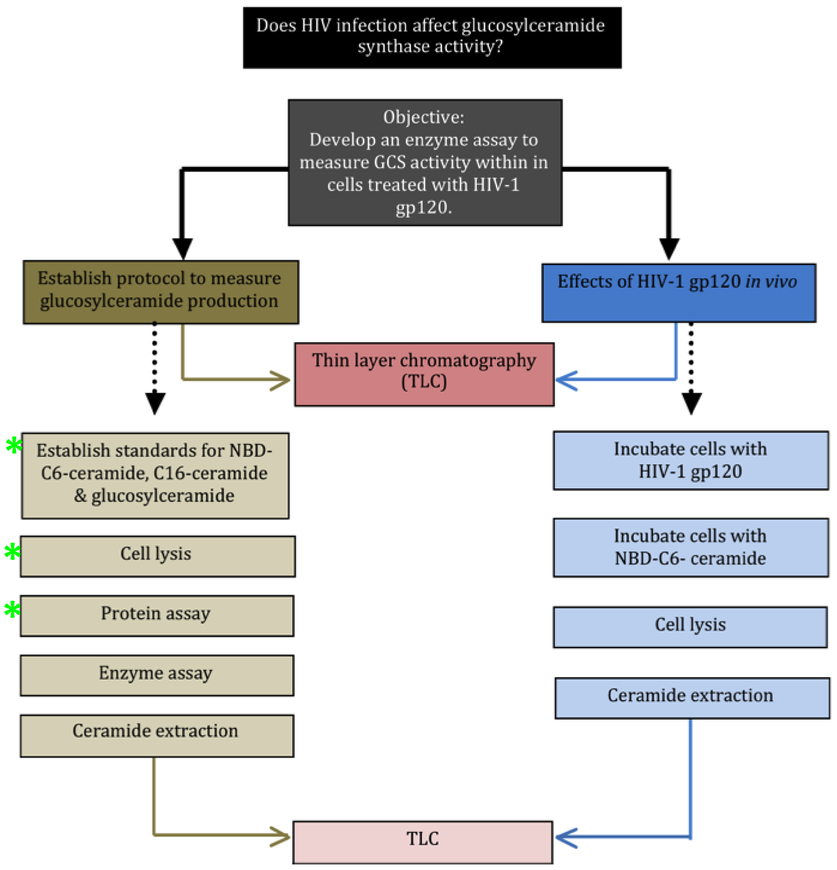
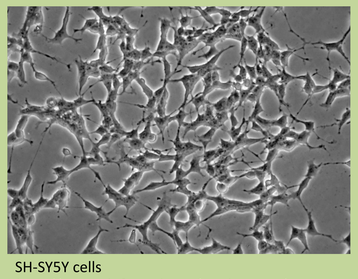


Figure 1. A flow chart of the methods needed to establish gluosylceramide synthase enzyme assay and test the effects of HIV-1 gp120 on enzyme activity. * represent completed in the development of the assay.

Methods

Tissue Culture

I performed tissue culture with SH-SY5Y neuroblastoma cells to test GCS activity in this cell line. The cells were grown to 80%-90% confluence in a 75cm^2 tissue culture flask. For use in fluorescent microscopy, cells were grown on 15mm tissue culture treated glass cover slips to a density of 5x10^4. For whole cell lysates, cells were grown in 6 well–tissue culture treated multi-well plates to a density of 5x10^5.



SH-SY5Y cells
http://www.qb.fcen.uba.ar/nicolaspregi/

Preparation of whole cell lysates

Monolayer cells in six well plates were lysed using Lysis 250 buffer. The cells remained on ice throughout the cell lysis procedure. Whole cell lysates were stored at -80° C until their use in protein assays or enzyme assays.

Determining protein concentration

Protein concentration was determined in whole cell lysates using Micro BSA™ Protein Assay Kit (Pierce).

Figure 2. Protein assay standard curve

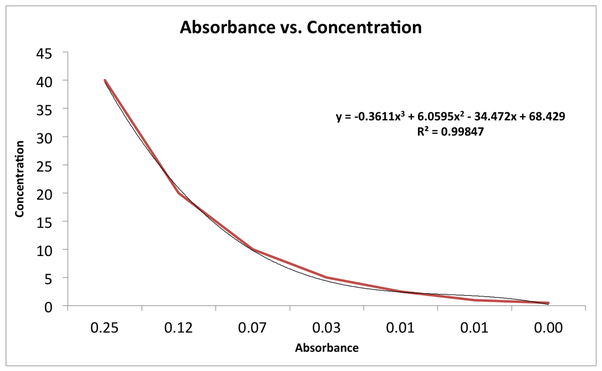


Figure 2. A representative standard curve of absorbance vs concentration of bovine serum albumin (BSA) protein. The red line is the standard curve and the black line is the line of best fit for the curve. The equation is the line of best fit for the standard curve.

Developing Thin Layer Chromatography Ceramide Standards

NBD-C6-ceramide, C16-ceramide, and glucosylceramide, (purchased from Cayman Chemicals (C6-ceramide) and Avanti Polar Lipids, respectively) were run on silica gel 60F plates. The lipids were loaded onto a TLC using the solvent system chloroform/acetone/methanol/acetic acid/water (10:4:3:2:1 ratio). Two methods of development were tested: 10%$CuSO_4$ in 8%H_3PO_4 with charring and iodine vapor. Iodine vapor was chosen as the best method to develop the plates.

Figure 3. Thin layer chromatography standards

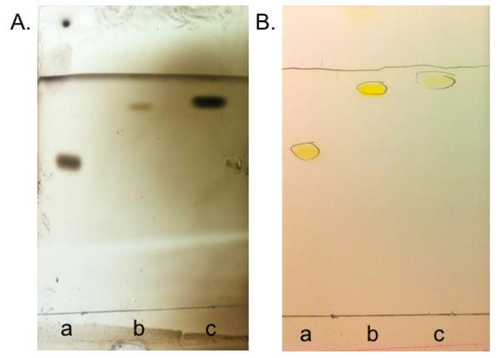


Figure 3, Results of thin layer chromatography of ceramide standards testing different developing methods. Fig. 3A. Development with 10%$CuSO_4$ in 8%H_3PO_4 with charring. Fig. 3B. Development with iodine vapor. Lanes a, b, and c represent glucosylceramide, NBD-C6-ceramide, and C16 ceramide standards, respectively. Rf values are as follows: Fig. 3A. a-0.65, b-0.86, c-0.88. Fig. 3B. a-0.67, b-0.92, c-0.95.

Discussion

With the enzyme assay developed, future experiments will answer whether or not HIV-1 gp120 negatively affects GCS. I theorize that either GCS is not functioning properly due to HIV-1 gp120 or ceramide is produced in such a high concentration that GCS activity can't keep up with the production.

References

- Campbell, S.M., S.M. Crowe, and J. Mak. "Lipid Rafts and HIV-1: From Viral Entry to Assembly of Progeny Virions." *Journal of Clinical Virology* 22.3 (2001): 217-227.
- Verma, S. P. "HIV: A Raft-Targeting Approach for Prevention and Therapy Using Plant-Derived Compounds (Review)." *Current Drug Targets* 10.1 (2009): 51-59.
- Vieira, Flavie S., Gladys Correa, Marcelo Einicker-Lamas, and Robson Coutinho-Silva. "Host-cell Lipid Rafts: A Safe Door for Micro-organisms?" *Biology of the Cell* 102.7 (2010): 391-407.
- Uchida, Yoshikazu, Satoru Murata, Matthias Schmuth, Martin J. Behne, Jeong D. Lee, Shinichi Ichikawa, Peter M. Elias, Yoshio Hirabayashi, and Walter M. Holleran. "Glucosylceramide Synthesis and Synthase Expression Protect against Ceramide-induced Stress." *Journal of Lipid Research* 43.8 (2002): 1293-1302.