Effects of N-linked Glycans on Fibrinogen Turbidity Assay

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Abstract

The goal of the fibrinogen turbidity assay is to study the effects of fibrinogen glycosylation on blood clot formation and structure and to observe fibrin polymerization over time. There are three important data results that could give us an insight on morphology and kinetics of fibrin networks:

- Lag phase
- Slope
- Maximum absorbance

Introduction

Fibrinogen characteristics:

- 340 kDa homodimer molecule
- 3 pairs of α, β, γ chains
- Four identified N-glycosylations (BN364, γN52)

- Fibrin polymerization occurs through cleavage of fibrinopeptides A and B by thrombin, then "knob-hole" interactions

Introduction

Methods

In order to observe the effect of glycosylations, different enzymes were used used to deglycosylate fibrinogen:

- Neuraminidase, cleaves off the terminal sialic acids from the carbohydrate chains
- PNGase, cleaves off the carbohydrate entirely by converting asparagine residue into aspartic acid

PNGase treated fibrinogen:

- Highest max absorbance, rate of fibrin polymerization and shortest lag time across both concentrations
- Neuraminidase treated fibrinogen:

- Longest lag phase and slowest rate of fibrin polymerization with both concentrations
- Higher max absorbance when compared to the WT, but smaller when compared to PNGase treated sample

Results

Turbidity assay results indicated changes in all three aspects depending on the type of deglycosylation as shown in the graph and table below:

<table>
<thead>
<tr>
<th>Deglycosylated Fibrinogen</th>
<th>Lag Phase (min)</th>
<th>Max Abs. 260 nm (AU)</th>
<th>Rate (nM/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT 0.8 mg/mL</td>
<td>65</td>
<td>0.350</td>
<td>0.000000000064</td>
</tr>
<tr>
<td>WT 0.4 mg/mL</td>
<td>65</td>
<td>0.160</td>
<td>0.0000000000109</td>
</tr>
<tr>
<td>PNGase treated 0.8 mg/mL</td>
<td>45</td>
<td>0.797</td>
<td>0.0000000000897</td>
</tr>
<tr>
<td>PNGase treated 0.4 mg/mL</td>
<td>45</td>
<td>0.077</td>
<td>0.00000000000090</td>
</tr>
</tbody>
</table>

Discussion

Both deglycoselated samples showed higher maximum absorbance than the WT which can indicate:

- Formation of thicker fiber or a denser fibrin network when compared to the WT
- The lag phase was the shortest with the PNGase treated sample, indicating removal of carbohydrates entirely speeds up formation and aggregation of protofibrils. The opposite effect was observed with the neuraminidase treated sample.

- The initial rate of kinetics was higher with the PNGase treated sample, and lower with the neuraminidase sample, giving us an insight on fibrin polymerization.

Acknowledgements

Funded by: R15HL150666

Dr. Nathan A. Hudson, Dr. Adam R. Offenbacher, Taylor Dement