Investigation of Enzyme Inhibition Mechanism

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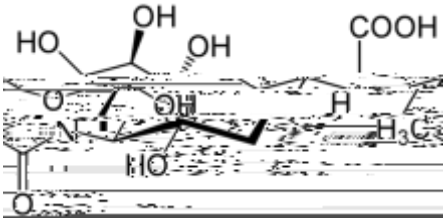


enzyme themselves, affecting the ability of the molecule to catalyze the specific reaction, without affecting substrate binding. Experimentally, this can be observed as a change in the maximal rate of reaction of the enzyme (Vmax). Finally, uncompetitive inhibition occurs as the inhibitor interacts with the enzyme-substrate complex, resulting experimentally, in changes in both substrate binding and maximal velocity (Km & Vmax). The nature of inhibitor action can be determined using a plot of reaction rates as substrate concentration is varied, allowing evaluation of the reaction kinetic variables.

Initial experiments on enzyme kinetics and inhibition, including substrate-velocity experiments, and calculations of Km, Vmax and Ki in a competitive-inhibitory system were carried out using rat cytosolic alcohol dehydrogenase, and pyrazole as an inhibitor. Because of difficulties anticipated in working with transferrin, and transferrin sialyltransferase, the model system chosen was a rabbit cytosolic lactose sialyltransferase. In this system, the sialyltransferase catalyzes the sialylation of lactose, yielding sialyllactose, in a manner analogous the sialylation of transferrin, as shown in Figure 2, below.

Figure 2:

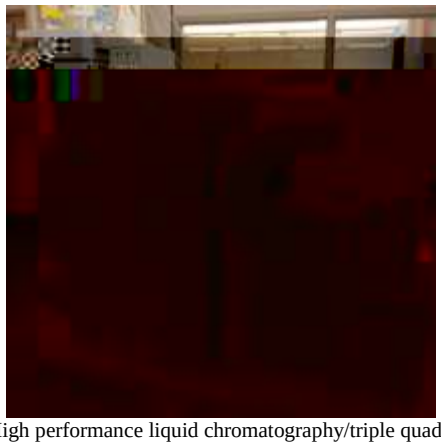


Figure 3: High performance liquid chromatography/triple quadrupole mass spectrometry instrument, located in Connecticut State Crime Laboratory

Figure 4: High performance liquid chromatography instrumented, located in Forensic Science department

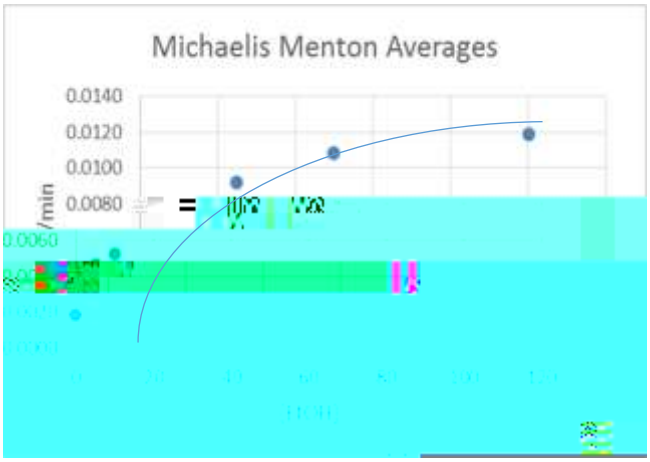
Results

Preliminary experiments using alcohol dehydrogenase as a basis for demonstrating competitive inhibition via substrate-velocity and Lineweaver Burk plots using rat cytosol were straightforward, and without complication. The analogous experiments with cytosolic sialyllactose are awaiting final analysis because of time and instrument issues with the Triple-Quadrupole Mass Spectrometer.

We have as yet failed to establish a method for the analysis of 3'-sialyllactose using high performance liquid chromatography. We are currently utilizing a gradient mobile phase system, transitioning from 10% acetonitrile to 50% acetonitrile in 10 mM ammonium acetate at 0.5 mL/min. Diode array detector was set at 250 nm, with 50 µl injection volume.

Discussion

Evaluation of an enzyme assay and determination of a classic Ki for pyrazole/alcohol dehydrogenase, performed as a training exercise for the evaluation of inhibition of sialyltransferase was achieved without



complication.

Figure 5: Example of Michaelis Menton plot with ethanol as an inhibitor of alcohol dehydrogenase

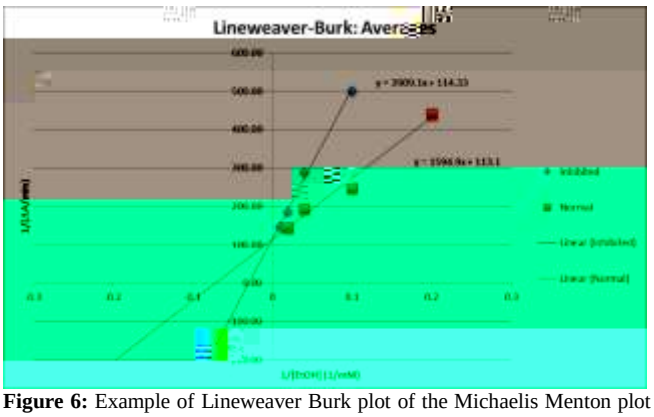


Figure 6: Example of Lineweaver Burk plot of the Michaelis Menton plot shown above

Competitive inhibition, as shown above in Figure 6, was showcased for this enzyme system. This was determined via analysis of Michaelis Menton kinetics, shown above in Figure 5, which lent its way to a Lineweaver Burk plot, shown in Figure 6, with intersection at approximately the y-axis. This intersection implies competitive inhibition. The two plots intersecting are a superimposition of the analysis let run with and without the inhibitor. The data from the analysis with the inhibitor is shown with the blue data points, and the uninhibited analysis is shown with the red data points.

Development of a direct analytical method for sialyllactose by HPLC in incubation samples was not resolved by the completion of the project. Nevertheless, the remaining analytical issues are expected to be resolved, allowing direct analysis of incubation samples by HPLC. Similarly, we are awaiting results from LC-MSMS instrumentation. Although these results will not be available for the completion of this portion of the research, they will

ideally become available for the next portion of this research. While routine analysis of incubation samples is expected to be performed on HPLC, the combination of HPLC and triple quadrupole mass spectrometry (LC-MSMS) allows for a higher degree of sensitivity, which may be important during the process of assay optimization.

Conclusions and Future Work

In this study, we have failed to develop a method for analysis of sialyllactose and components of an enzymatic assay using solely high performance liquid chromatography. There is a possibility that these components may be detected using high performance liquid chromatography/triple quadrupole mass spectrometry, however, that information is not available at the time of completion of this paper. Finally, a proper understanding of