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1H-NMR and LC/MS assay development for the characterization of glycosidase and glycosyl transferase activities of MalA from Bdellovibrio bacteriovorus

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^{1}H-NMR and LC/MS assay development for the characterization of glycosidase and glycosyl transferase activities of MalA from *Bdellovibrio bacteriovorus*

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Introduction

Sequencing of the predatory bacteria *Bdellovibrio bacteriovorus'* genome in 2005[1] revealed three potential carbohydrate-active genes, including a putative maltase MalA, which were unexpected given *Bdellovibrio's* observed disuse of prey carbohydrates.[2] In order to understand MalA's function in the life of the potentially useful living anti-biotic *Bdellovibrio,* the native substrate and activity of the enzyme must be determined.

Determination of activity in glycolytic enzymes has traditionally been done using a glucose oxidase colorometric quantitation of glucose.[3,4] This method is limited to determining the cleavage of glucose from longer oligosaccharides and does not allow for characterization of other possible enzymatic activities, including glycosyl transferase activity, which has been observed in MalA by qualitative TLC experiments.[2]

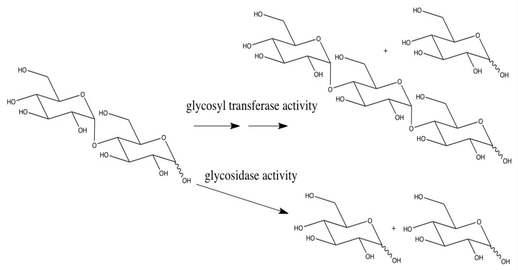

Figure 1. Possible activities of carbohydrate-active enzymes on maltose.

In order to characterize the possible activities of MalA, we developed a convenient, qualitative 1H-NMR method for continuously assaying the relative presence of glucose, maltose, and maltotriose in solution with MalA.

Recent studies have quantitated small carbohydrates, typically monosaccharides, by liquid chromatography coupled to electrospray ionization (ESI) mass spectrometry (LC/MS) utilizing low-sample handling carbohydrate-alkali metal adduct ionization techniques.[5,6] We aimed to extend the quantitation of glucose by cesium cation attachment to longer maltooligosaccharides for analysis by ion trap mass spectrometry. The ability to quantitate underivitized maltooligosaccharides in solution will allow for the rapid characterization of carbohydrate-active enzymes on a variety of substrates, and will facilitate enzyme-substrate interaction studies.

1H-NMR method

• 1H NMR was used to assess the relative concentrations of glucose and two maltooligosaccharides (α,1-4 linked glucose polymers) by analyzing the anomeric region of 1H NMR spectra.

•The anomeric proton in maltooligosaccharides (and glycosidic protons in DP > 1), as a hemiacetal (or glycosidic acetal), has a chemical shift significantly higher than any other proton. It appears with different shift depending on its anomeric configuration (Figure 2).

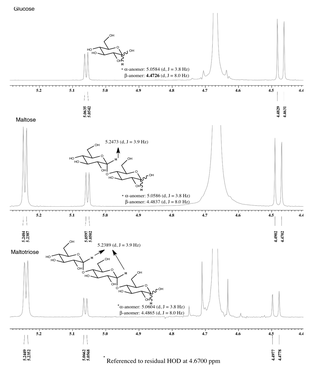

Figure 2. Determining maltooligosaccharides by 1HNMR.

1H-NMR results

The three studied sugars were found to be distinguishable by the β-anomer resonance (glucose from maltose and maltotriose), or the glycosidic proton resonance (maltose from maltotriose).

The above method was applied to a continuous assay of MalA's activity on glucose, maltose, and maltotriose. MalA is active on maltose and maltotriose, and shows no activity on glucose. Some glycotransferase activity is seen by the accumulation of maltotriose in the presence of maltose (Figure 3).

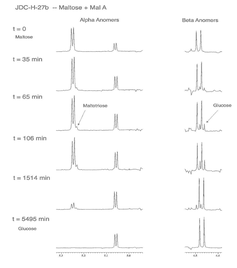

Figure 3. Continuous assay of MalA's activity on maltose shows glycotransferase activity by the accumulation of maltotriose.

LC/MS method

•All experiments were conducted on an Agilent 1100 series LC/MSD SL Ion Trap with an electrospray ionization (ESI) ion source.

•Pseudo-molecular ions $[M+Cs]^+$ were formed by addition of 40 µM cesium acetate in the 80/20 acetonitrile/water mobile phase.

•The trap was optimized to maximize transmittance of the $[Maltose+Cs]^+$ pseudo-molecular ion (m/z = 475).

•Typically 5 µL of sample, dissolved in water or the mobile phase, was injected for each measurement.

•No chromatographic separation is currently in place.

LC/MS results

Pseudo-molecular ions $[M+Cs]^+$ were observed for glucose, maltose, and maltotriose (Figure 4).

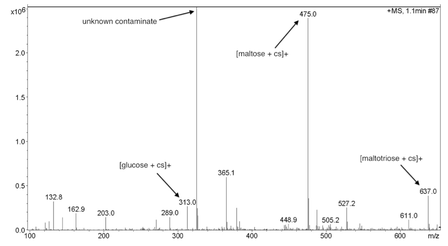

Figure 4. Mass spectrum of glucose/maltose/maltotriose mixture with cesium acetate in mobile phase.

Technical difficulties prevented rigorous method verification, but preliminary results suggest that the three studied sugars (DP1-3) should be quantifiable from at least 1 to 1000 ng per injection (180 nM - 1.1 mM) using 40 uM cesium acetate in the mobile phase (Figure 5).

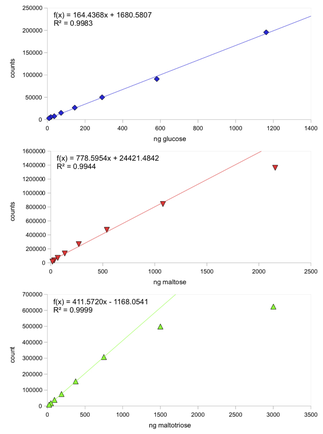

Figure 5. Quantitation of underivatized maltooligosaccharides by Cs+attachment using LC/MS.

Conclusions

1H-NMR studies on MalA

•1H-NMR is an efficient continuous method for qualitatively screening glycosidase and glycotransferase activities on small carbohydrates.

•Glycolytic activities in MalA on maltose and maltotriose, but not on trehalose (α- (1→1) glucose dimer) were observed by 1H-NMR.

• Maltotriose was seen to accumulate from maltose in the presence of MalA. This supports earlier observations of glycosyl transferase activities seen by TLC.[2]

Quantitation of carbohydrates by LC/MS

•The LC/MS method has high potential for easily quantitating underivitized carbohydrates in solution, allowing for high-throughput screening of carbohydrate-active enzyme activities on various substrates.

•We should be able to precisely determine relative carbohydrate concentrations in solution in the range of roughly 1-1000 ng of each carbohydrate per 5 µL sample with a method time under 10 minutes per sample.

•This means potentially **detecting 0.1% or less changes in the carbohydrate composition of a solution**, making detection by cesium attachment in LC/MS an ideal method for assaying carbohydrate-active enzymes.

Future work

•Thorough method verification and optimization will be conducted for the three carbohydrates currently under study. We should be able to apply the same methodology to longer oligomers.

•Apply the method to determine relative concentrations of sugars in a mixture.

•Employing an amino or cyclodextrin stationary phase HPLC column for carbohydrate separation should allow for improved sensitivity. Properly separated samples should elute in shorter time periods, effectively concentrating ions for easier detection. With carbohydrates separated according to degree of polymerization, we can optimize ion transmission for each carbohydrate in solution, maximizing the sensitivity potential of the ion trap. Finally, separation will allow for fragmentation studies, which have been used in quantitation,[5] and can provide structural information for unknown analytes.

•Continue organic syntheses toward small carbohydrate derivatives for kinetic studies with MalA, which will lead to insights on substrate-enzyme interactions.[7]

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For further information

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