

# Development of Nano-Scale Amide Exchange-MS to Study Native Enzyme-Carbohydrate Systems

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## OVERVIEW

Evaluating a Nano-Scale Amide Exchange MS apparatus using Angiotensin-I



## INTRODUCTION

The interactions between pectin degrading enzymes excreted by various plant pathogen and plant cell wall carbohydrates are of significant importance both industrially and agriculturally. Due to the hydrolytic activity of pectinases, a mutant form of endopolygalacturonase-II (EPG-II) from *A. niger* will be used so that it would remain bound to the oligomeric substrate for the duration of the deuterium exchange procedure.

We would like to explore the possibility of using wildtype EPG-II, rather than the mutant, if the procedure is carried out in a large excess of substrate.

Oligosaccharides must be collected from natural sources, because there is no current synthetic alternative, making large excesses difficult and expensive to achieve.

Having miniaturized the apparatus the system was first evaluated using Angiotensin-I (MW = 1296). Amide exchange-MS miniaturized scale capillary-LC and picomoles of the sample was used.

The system will then be applied to the study of Enzyme-Carbohydrate interactions without the use of a Mutant Enzyme and femtomoles of protein.

## APPARATUS

The LC apparatus has been adapted to combine the necessary operational deuterium exchange conditions with the capillary-LC.

Along with miniaturizing the system with nanospray tubing capillary columns lower flow rates required for nanospray-MS

Necessary to decrease the analysis time to reduce the possibility of back exchange.

## METHOD

- For analysis of Angiotensin-I (Fully Deuterated)
  - 0.6ul of 1.0mg/ml
  - Diluted by 100 in deuterium
    - concentration = 10pmol/ul
  - Exchange:
  - Dried down using speed vac
    - Deuterium added 24 hr prior to the addition of KCl and pepsin
  - End of incubation, tube
    - ice-bath
    - 6.0ul of 0.1M HCl added, quenched
    - 2.4ul of 0.1mg/ml pepsin, digestion
  - Digested
    - 2 days
- For the analysis of Angiotensin-I (non deuterated)
  - The only difference in the method was, deuterium was added along with the acid and pepsin

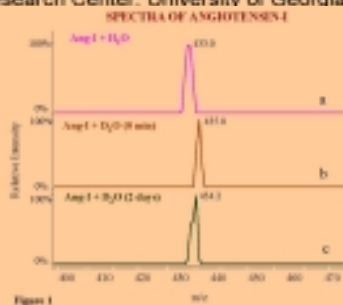


Figure 1

- The peptide 433 m/z unit corresponds to M+3H peak of Angiotensin-I
- Spectrum a: Angiotensin-I in H<sub>2</sub>O, and used as a control
- Spectrum b: Angiotensin-I in D<sub>2</sub>O after 24 hr exchange, recorded at time = 0 min
- Spectrum c: Angiotensin-I in D<sub>2</sub>O after 24 hr exchange, recorded after 2 day digest period

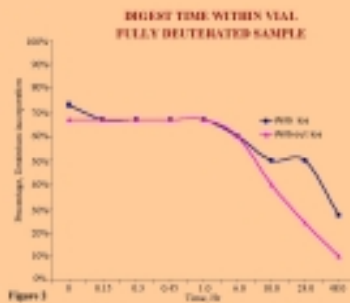


Figure 3

- The above figure shows the percentage of deuterium incorporation for a fully deuterated Angiotensin-I sample.
- Data points on graph corresponds to sample taken out periodically during digest and used for analysis with nanospray-MS

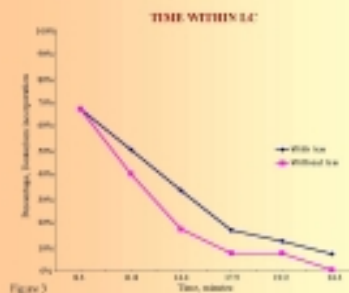


Figure 2

The % of D<sub>2</sub>O incorporation was found to decrease as the time within the LC increased

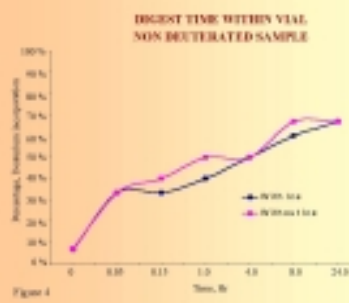


Figure 4

For analysis of Angiotensin-I (non deuterated sample) the % of D<sub>2</sub>O incorporation was found to increase with digest time.

## CONCLUSIONS

- It can be concluded from the digest time in vial, that the digest can be carried out for a longer period of time since limited back exchange takes place
- Hence allows us to use lesser amount of enzyme, thus decreasing the background from pepsin catalyzed products
- From time in LC it can be said that,
  - Longer the gradient time, the more deuterium is lost
  - Different levels of deuterium retention can be obtained depending on when the peptide elute from the column

## FUTURE WORK

- System can be applied to the study of enzyme-carbohydrate interactions without the use of mutant enzyme
- Interested in the interactions between pectin degrading enzymes excreted by various plant pathogen and plant cell wall carbohydrates

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