The Search for Polygalacturonase Inhibiting Proteins

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Overview

- Identification of polygalacturonase inhibiting proteins (PGIPs) through the use of SPR Biosensors and tandem mass spectrometry
- The interaction of plant cell wall derived PGIPs with immobilized polygalacturonases is detected on a Biacore 3000, and the corresponding PGIP's retrieved for analysis
- Sequence information pertaining to the retrieved PGIP is generated using a Micromass Q-ToF tandem mass spectrometer

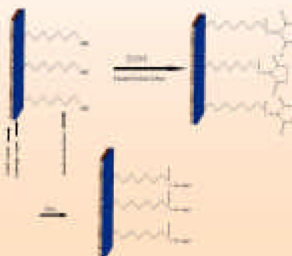
Introduction

Polygalacturonase inhibiting proteins found in plant cell walls are known to inhibit the destruction of the cell wall caused by the release of fungal enzymes known as polygalacturonases. Inhibition begins when a PGIP binds to a PG forming a PGIP/PG complex. Due to these interactions we are able to isolate and retrieve PGIPs from a plant cell wall preparation using a SPR biosensor. Sequence information from the retrieved PGIP is then generated using tandem mass spectrometry. The sensitivity of the SPR biosensor allows us to confirm the formation of PGIP/PG complexes, and the high sensitivity of the mass spectrometer allows us to generate sequence information pertaining to the interacting PGIPs.

Methods

Binding of PGIP/PG complexes are achieved on a Biacore 3000 biosensor. Known PGs from *Aspergillus niger* are mixed with 10mM sodium acetate at pH 5.5 for optimum binding and then immobilized on a Biacore CM5 sensor chip using a standard EDC/NHS protocol. Next a PGIP is extracted from the cell wall of *Phaseolus vulgaris* (Bean) by affinity and ion exchange chromatography, mixed with a 100mM sodium acetate buffer pH 5.0, passed over the sensor chip surface and coordinating PGIPs are retained. The bound PGIP is then eluted from the sensor chip, digested with trypsin and analyzed using a Micromass Q-ToF tandem mass spectrometer.

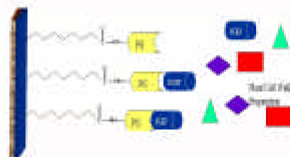
Immobilization Chemistry



Immobilization

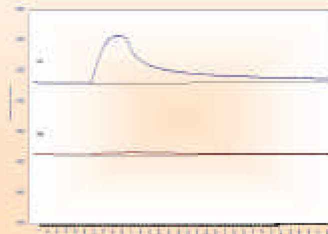
- A) Baseline
- B) EDC/NHS activation
- C) Covalent coupling of ligand
- D) Deactivation of unbound dextran by ethanolamine
- E) Final amount immobilized
  - Immobilization is maximized for good isolation experiments

PGIP Capture



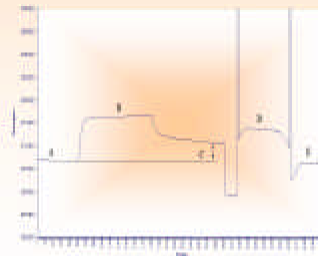
Indication of Specific Binding

- PGIP only binds to a surface with bound PGB. This tells us that binding is due to the interactions of the PGIP with the PG and not with the chip surface
- A) Blank surface - all reactive sites blocked with ethanolamine
- B) Binding of PGIP to PGB



Microrecovery of Known PGIP from a Pure Sample

- A) Baseline
- B) Binding of PGIP
- C) Amount of PGIP bound
- D) Injection of recovery buffer
- E) Original baseline recovered - All bound PGIP recovered



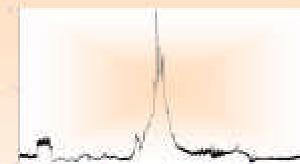
Tryptic Digest of Recovered Sample

- Sample was digested with porcine modified sequencing grade trypsin for 36 hours at 37°C
- Millipore® ZipTip$_{C18}$ was used to concentrate and purify the digested sample

Capillary LC/MS/MS

- Recovered sample was introduced into the Q-ToF (Micromass) mass spectrometer using a Waters CapLc. The original CapLc solvent delivery configuration was modified to minimize the dead volume between the mixer and the nanocapillary column.
- The mobile phases used for gradient elution consisted of (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid.
- A gradient of 10% B to 70% B over 70 minutes at a flow rate of 0.160 µL/min was used to elute the peptides.
- The instrument was operated in a data dependent scan mode.
- The survey MS spectra were acquired from 400-1700.
- The switch criteria for MS to MS/MS mode were ion count and charge state.
- The instrument was set to ignore singly charged ions and to acquire MS/MS data for up to 3 co-eluting peaks. The collision energy was varied depending on the peptide mass and charge state.

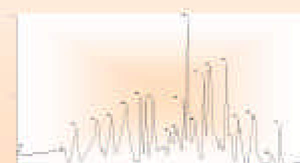
TOF MS Survey Total Ion Chromatogram



MS/MS Component 1

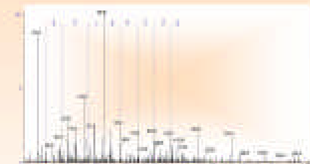
MS/MS Component 2

MS/MS Component 3



Examples of Peptide Sequences obtained from MS/MS data

- A leucine rich repeat motif is indicative of a PGIP
- Only the Y ions are labeled



Conclusion

- PG was successfully immobilized onto the Biacore CM5 chip surface
- The coordinating PGIP bound to the PG and was recovered for further analysis
- Several peptide sequences containing leucine rich repeats indicate the presence of PGIP

Acknowledgements:

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