On-tissue Digestion using Microwave for MALDI Imaging

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Material
- Protein: horse radish peroxidase (HRP), bovine serum albumin (BSA), and cytochrome C ( Sigma, Stein)
- Trypsin (porcine, modified sequencing grade, Promega)
- Chemicals: ammonium bicarbonate, diethanolamine (DT, Isoquest, Stein) and tris(hydroxymethyl)aminomethane (Tri, Sigma, Stein)
- Solvents: acetonitrile, and methanol ( Merck)

Introduction
Enzymatic digestion for protein identification or glycan separation is a critical step in proteomics or glycomics research. However, it is time consuming, often requiring several hours to overnight. Moreover, it is difficult to digest tissue in situ for MALDI imaging. Microwave assisted enzymatic digestion has been shown to be very efficient in solution digestion or in gel digestion. It decreases digestion time to several minutes for trypsin digestion in solution. We have applied this method on tissue sample for MALDI imaging. Mouse brain tissue has been used as a sample tissue and standard proteins such as BSA and cytochrome C on target have been used as a control example to obtain the optimum conditions for digestion.

Methods
- Protein Pattern with standard proteins on target has been prepared by a pico-titer emitting devices (MALDI picoto, HST).
- Microwave assisted digestion reaction was performed with time range of 1 to 30 min with variable power from 100W to 700 W.

Results
- In-solution digestion of HRP with microwave heating was completed within 3 min and gave protein digestion efficiency about 1.5 times higher than a conventional protocol.
- In-gel digestion took 35 min and higher peptide recovery efficiency could be achieved with microwave heating.
- For tissue, microwave induced digestion has been obtained and peptides peaks identified. More experiments to obtain the optimum conditions for microwave digestion will be performed.

Conclusions
- Microwave-assisted digestion (MAD) has enhanced both in-solution and in-gel digestion efficiency. MAD is useful for large scale proteomic research to reduce operating time.
- Microwave treatment also enhances digestion efficacy for tissue on a MALDI target.

References

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