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Application News

No. MO339

AXIMA Performance MALDI-TOF
AXIMA Resonance MALDI-QIT-TOF

Characterization of Early Stage Maillard Reaction Products using MALDI Mass Spectrometry and PTM finder™ Software

- Rapid analysis of glycated peptides using the AXIMA Resonance
- Identification of specific fragmentation patterns indicative of protein glycation
- Efficient screening for glycation site using the PTM Finder software

Characterization of Early Stage Maillard Reaction Products using MALDI Mass Spectrometry and PTM Finder™ Software

Introduction

The Maillard reaction (Maillard L-C 1912 Comptes rendus hebdomadaires des séances de l'academie des sciences 154 66-68) occurs non-enzymatically between the reducing end of a sugar (C=O) and a reactive NH_2 group of a protein. The detailed characterization of the reaction products is of high importance in the field of food processing but also in physiologies and pathologies of human diseases such as the chronic vascular complications of diabetes, Alzheimer's disease and ageing. In this study the reaction of human serum albumin (HSA) and standard peptides with glucose, maltose and lactose was investigated. The molecular mass of the whole glycated HSA and the total mass of sugars that reacted with the protein were measured. HSA was then digested in order to locate the reaction sites of each sugar. Modified peptides were fragmented using CID experiments in order obtain signatures at the MS/MS level.

Methods

HSA, substance P (Sub. P) and the three different sugars: glucose, maltose and lactose were purchased from Sigma (Poole, UK). The proteins were incubated at 50°C with various concentrations of sugars (0.5; 0.1; 0.05 and 0.025M) for 3 weeks in a bacteriostatic phosphate buffer. The proteins were purified using C_4 ZipTip® (Millipore, USA). Intact molecular weight measurements were acquired using an AXIMA Performance™ MALDI TOF/TOF instrument in linear positive ion mode. Trypsin digested proteins were purified/separated using a nano-LC Accuspot™ system and analyzed using both an AXIMA Performance™ MALDI TOF/TOF and an AXIMA Resonance™ MALDI QIT-TOF mass spectrometer in positive ion mode. The MS/MS spectra of the (modified) tryptic peptides were acquired using three different CID excitation energies allowing the selective fragmentation of the sugar moieties. The raw data were acquired using Shimadzu LC-MALDI software then processed in the PTM Finder™ package.

Results

The incubation of HSA and Sub. P with each of the sugars appeared to be complete after 3 weeks, with no further adduction of sugars seen after this time. The extent of non-enzymatic condensation of sugars was monitored using the AXIMA Performance™ in linear mode for protein detection and reflectron mode for peptide detection. Sub. P (RPKPQQFFGLM; MH^+ 1347 Da), glycation with glucose (MH^+ 1509 Da), maltose (MH^+ 1671 Da) and lactose (MH^+ 1671 Da) were easily detected by MALDI TOF analysis (Figure 1).

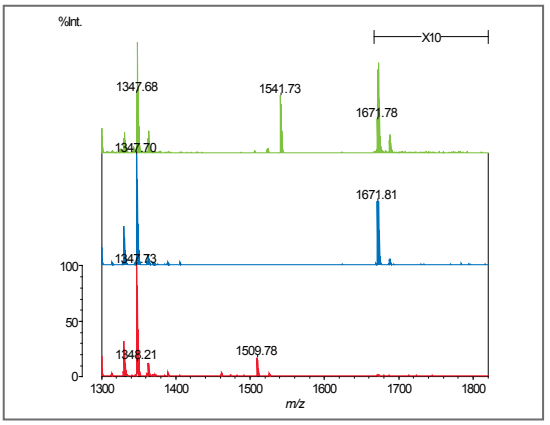

Figure 1: MALDI TOF analysis of Sub. P modified with glucose (red), maltose (blue) and lactose (green)

The control HSA was detected at approximately 66,410 Da. The reaction product of HSA with glucose exhibited a molecular weight of 69,290 Da which indicated the condensation of around 18 glucose molecules. The condensation reaction with maltose was similar to that of lactose and exhibited a modified protein molecular weight of 70.17 kDa, which would correspond to an adduction of 3761 Da or 11.6 sugar residues (Figure 2).

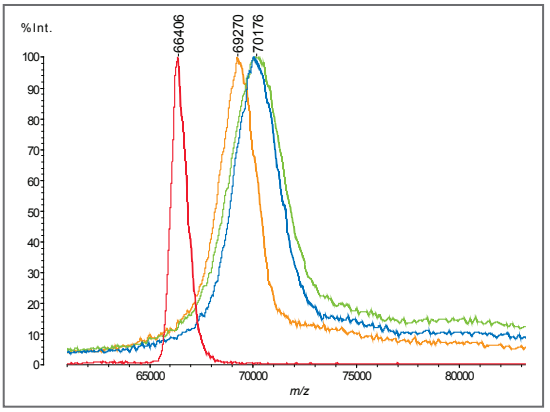

Figure 2: MALDI TOF analysis of native HSA (red) and HSA modified with glucose (orange), maltose (blue) and lactose (green)

The tryptic digests of each modified HSA and the control HSA were separated using a Prominence nano-LC system linked to an AccuSpot™ MALDI plate spotter for sample collection. The acquisition was performed using the LC-MALDI software to generate MS/MS spectra for each precursor. This data was then interrogated using the PTM Finder™ software. PTM Finder™ is a Shimadzu proprietary software that uses data mining to investigate post-translational modifications, including data for hypothetical, novel modifications. The PTM Finder™ software uses initial Mascot® (Matrix Science, UK) search results to generate a restricted protein database consisting solely of the matched proteins from those searches. Any remaining un-matched precursors submitted to Mascot® through the PTM Finder™ software (Figure 3) can then be screened for either labile or non-labile user-definable modifications using the PTM Define function.

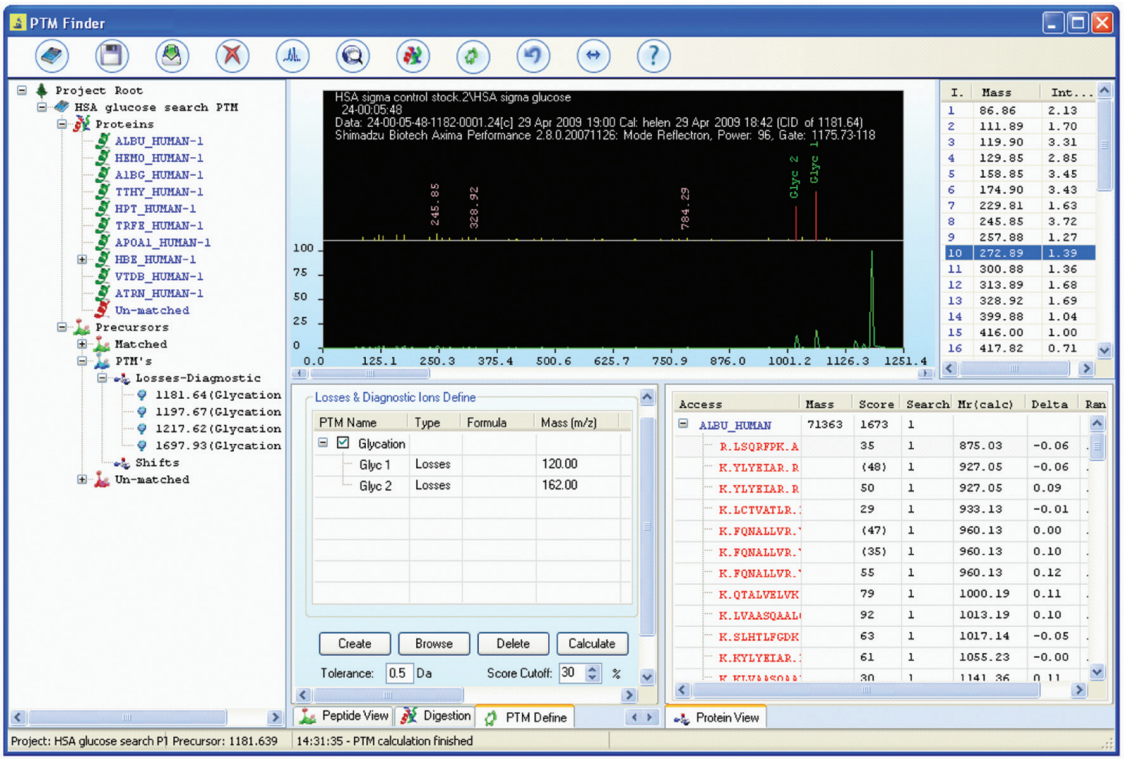

Figure 3: PTM Finder ™ software

The control and modified HSA tryptic digest LC-MALDI data were initially processed through integrated Mascot® and the results for each protein are shown in Figure 4. Then, using the PTM Define function, the specific MS/MS signature for glucose glycation was screened against the LC-MALDI data. Glycation is a labile modification under UV MALDI conditions and glucose glycation has previously been shown to generate a reproducible pattern in MS/MS of mass losses from the precursor (Loss of 36, 120 and 162 Da from the precursor.) (Brancia F.L. et al J. Mass Spectrom. 2006; 41: 1179-1185). Similar patterns have been determined for maltose and lactose modified proteins. For this, the PTM Finder™ software was used to screen for the loss of the entire disaccharide (324 Da) from the un-matched precursors, thus highlighting possible lactose and maltose modification candidates. Each of these candidates was then fragmented using the AXIMA Resonance ™ to identify a specific loss pattern from the precursors indicative of either lactose or maltose. The three loss patterns for glucose, maltose and lactose modification are shown in Figure 5 and were determined to be highly reproducible for each given modification.

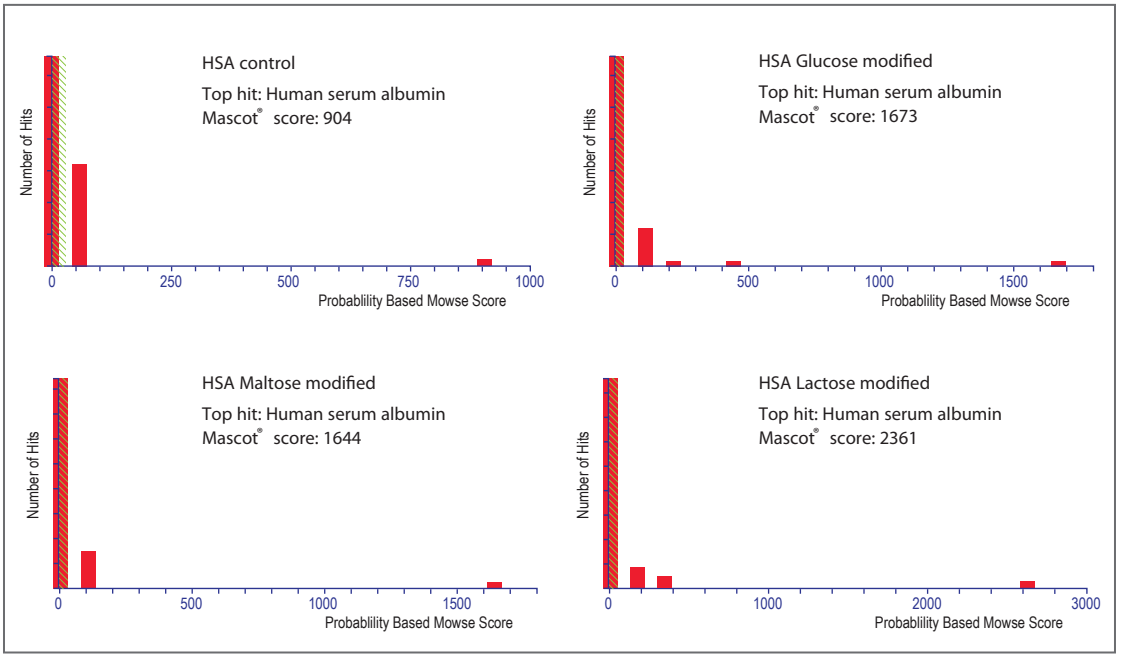

Figure 4: Search results for each modified protein using integrated Mascot® (Matrix Science, UK) within the PTM Finder™ software

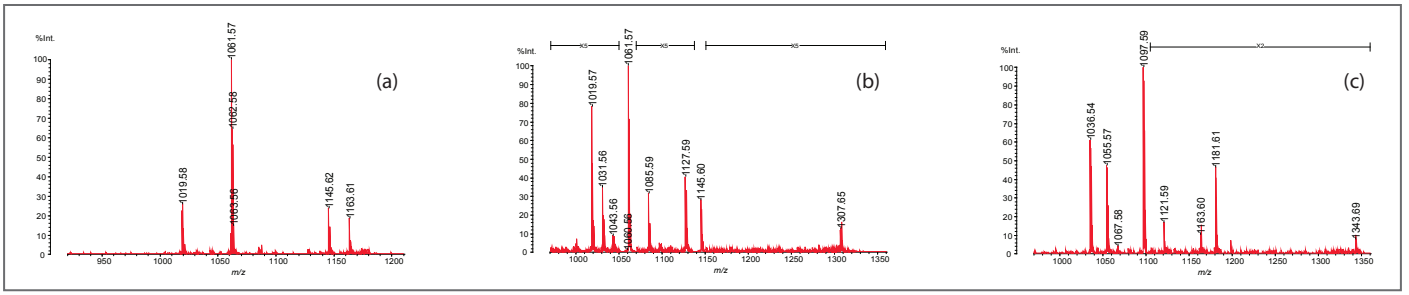

Figure 5: Characteristic MS/MS fragmentation patterns for glucose (a), lactose (b) and maltose (c) protein glycation

Interestingly, the CID experiments indicated that for a specific collision energy the glycated and native versions of a given peptide exhibited different MS/MS fragmentation under the same conditions. In particular, it was observed that the glucose, maltose and lactose modified tryptic peptides exhibited a favoured energetic pathway leading solely to the characteristic loss patterns discussed previously with virtually no further fragmentation. This hypothesis was extensively tested using three different CID energies (nominally 200, 350 and 500).The data shown in Figure 6 shows the comparison between the MS/MS fragmentation of the HSA peptide AFKAWAVAR (MH^+ = 1019.2 Da) and glucose glycated AFKAWAVAR (MH^+ = 1181.2 Da) using the different collision energies.

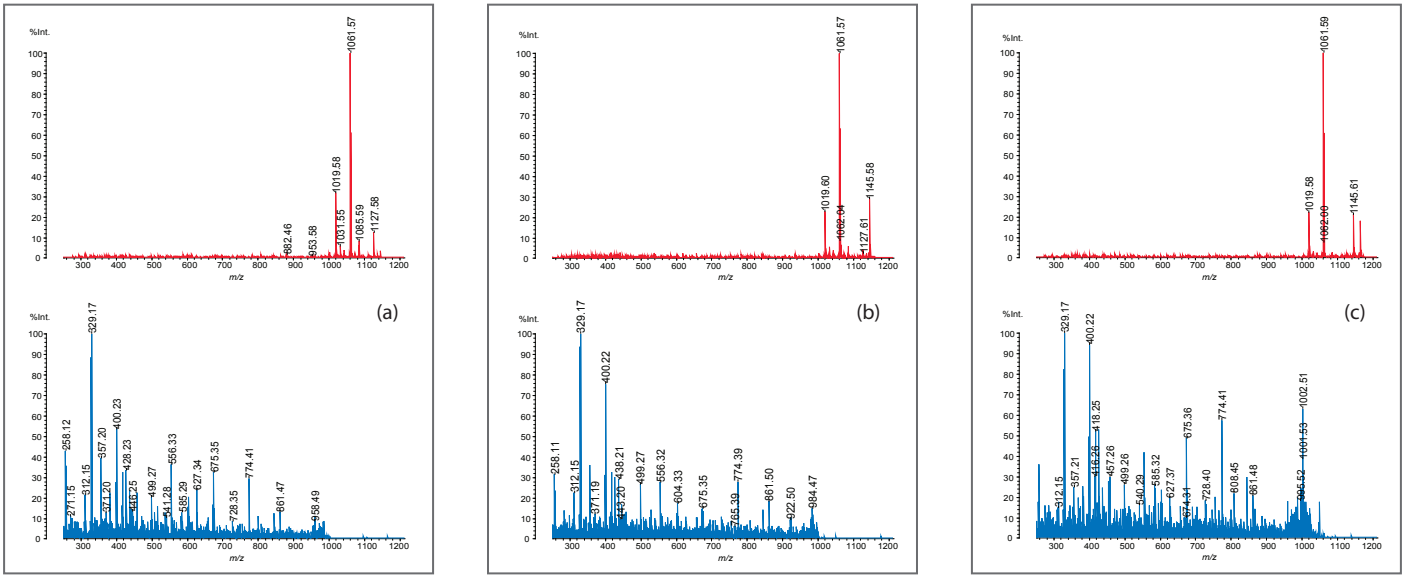

Figure 6: Difference in MS/MS fragmentation of the HSA peptide AFKAWAVAR (MH^+= 1019.2 Da; blue trace) and glucose glycated AFKAWAVAR (MH^+ = 1181.2 Da, red trace) for collision energy settings 200 (a), 350 (b) and 500 (c)

Conclusion

- The PTM Finder™ software provides a rapid and simple solution for PTM identification from MS/MS data
- Characteristic fragmentation patterns for glycated proteins using glucose, lactose and maltose have been determined
- A favoured pathway for MS/MS fragmentation of modified peptides has been demonstrated

Second Edition: August, 2013

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