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The study of aging processes using *in vitro* glycation experiments applied to low density lipoproteins using MALDI-QIT-TOF-tandem Mass Spectrometry

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Introduction

Due to the steadily increased human lifespan during the last centuries our society is increasingly faced with all sorts of health problems related to "aging processes" that are now causing a huge stress on the healthcare system of any modern country. Today glycation of proteins also known as "Maillard reactions" is increasingly recognized to be related to aging processes involved not only in the development of human disease but also in altering the quality of food and pharmaceutical formulations [1, 2]. In this context, the modification of low-density lipoprotein (LDL) induced by elevated glucose levels in combination with reactive oxygen species (ROS) is considered a major risk factor of cardiovascular disease (CVD) and atherosclerosis [3, 4]. In order to track the range of modifications at the molecular level we have developed a methodology to specifically detect glycation sites on various standard proteins in order to be able to look at real samples such as human serum albumin (HSA), immunoglobulins (e.g. IgG) and lipoproteins (e.g. LDL) *in vivo* [5]. Our lipoproteomics approach using (LC-)MALDI-QIT-TOF-MS/MS combined with an intelligent software tool (PTM Finder™, Shimadzu, UK) represents a unique platform for studying lipids and modification of amino acids (AA) in proteins on the molecular level.

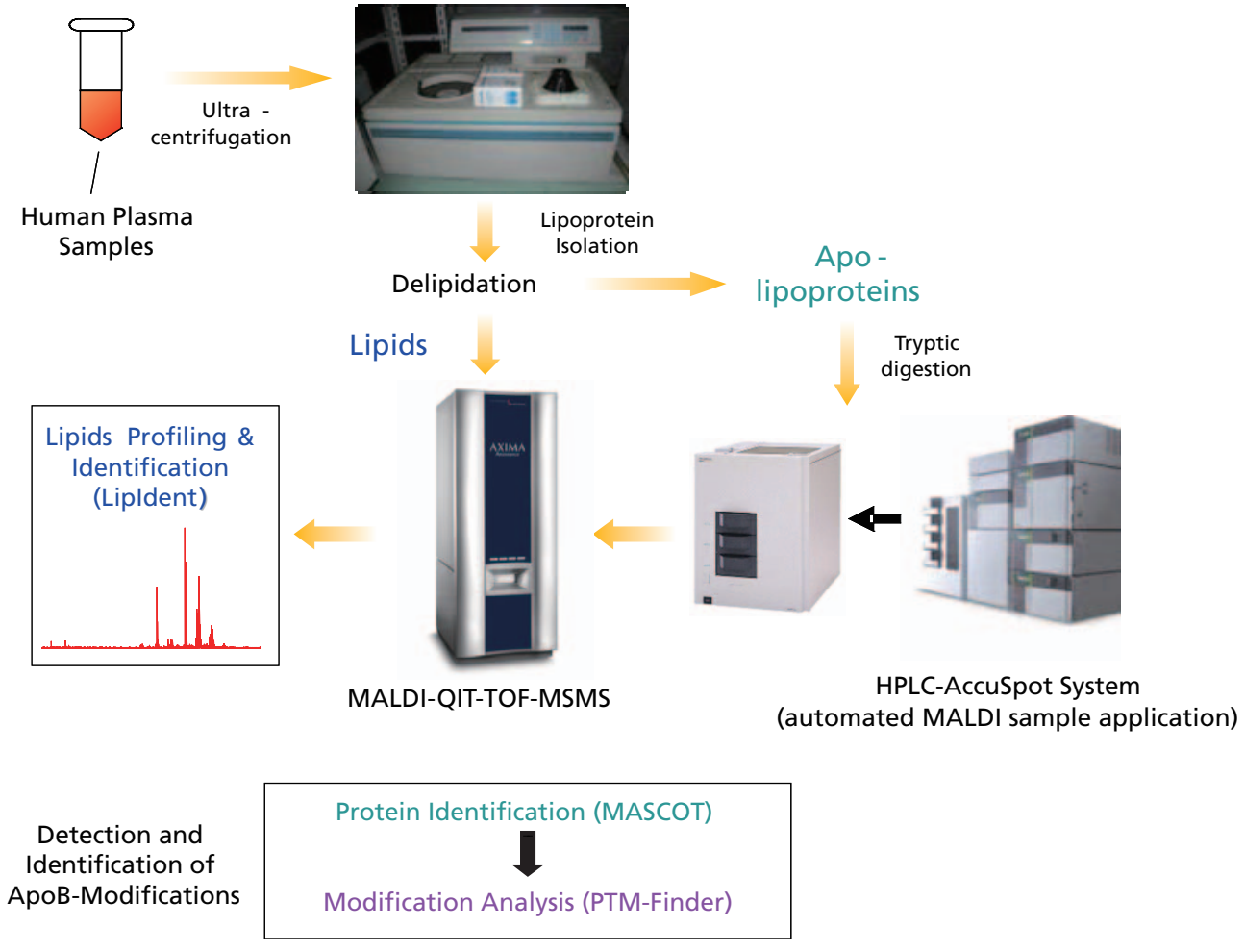

Fig. 1 Workflow of the MALDI-QIT-TOF-MS/MS based lipoproteomics approach

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Experimental

Lipoproteins were isolated from normal human plasma by preparative ultracentrifugation and modified by diverse ROS (e.g. Cu^{2+}, HOCl, etc) and lipid peroxidation products (e.g. MDA, HNE) in the presence or absence of sugars (e.g. glucose). PLs were extracted and directly analyzed by MALDI-MS. The apolipoproteins were digested with endoproteinases (e.g. trypsin), peptides were separated by HPLC and sequence mapping was performed based on MALDI-MS/MS. Mass spectra were recorded by MALDI-TOF-RTOF- and MALDI-QIT-TOF-MS/MS using AXIMA-Performance and AXIMA-Resonance (Shimadzu, UK), respectively. Lipids were analyzed using THAP in 80/20; MeOH/100mM DAHC (v/v) and for peptides CHCA (5 mg/ml) in 50/50 ; ACN/0.1% TFA (v/v) containing 10mM ADHP was used [6, 7]. Data were processed and searched for specific MS/MS signatures based on a parameterizable database and software module (PTM Finder, Shimadzu). Lipids are identified and analysed using prototype software tools (LipIdent) developed in cooperation with external partners. An overview of our workflow is provided in Fig. 1.

Results

Phospholipids (PLs) were recorded in positive ion MALDI-TOF-MS mode in the *m/z* range 400-900 and MALDI lipid profiles of the different *in vitro* modified LDL particles were established (Fig. 2). A decrease of PC containing sn-2 PUFA (i.e. 18:2, 20:4, 22:6) between 19-75%, and increase of LPC16:0/18:0 between 42-350% compared to nLDL was observed in oxLDL, gLDL, and goxLDL, respectively. This indicates for strong lipid degradation and enzymatic hydrolysis (e.g. Lp-PLA2 activity) during the LDL modification process initiated by ROS and/or glucose. MALDI-TOF-MS/MS spectra of peptides were acquired and large peptide MS/MS dataset (more than 400 MS/MS spectra per LDL type) were searched for characteristic neutral losses linked to sugar moieties attached to the amino acid backbone (-162, -120 and -36 Da) using the PTM Finder tool (Shimadzu, UK) (Fig. 3 and 4). Based on this approach distinct glycation sites located in vicinity of the LDL-R binding region of ApoB-100 were found in gLDL. Our finding may explain some of the biological dysfunction related to elevated plasma levels of glycated LDL species (e.g. in diabetic patients).

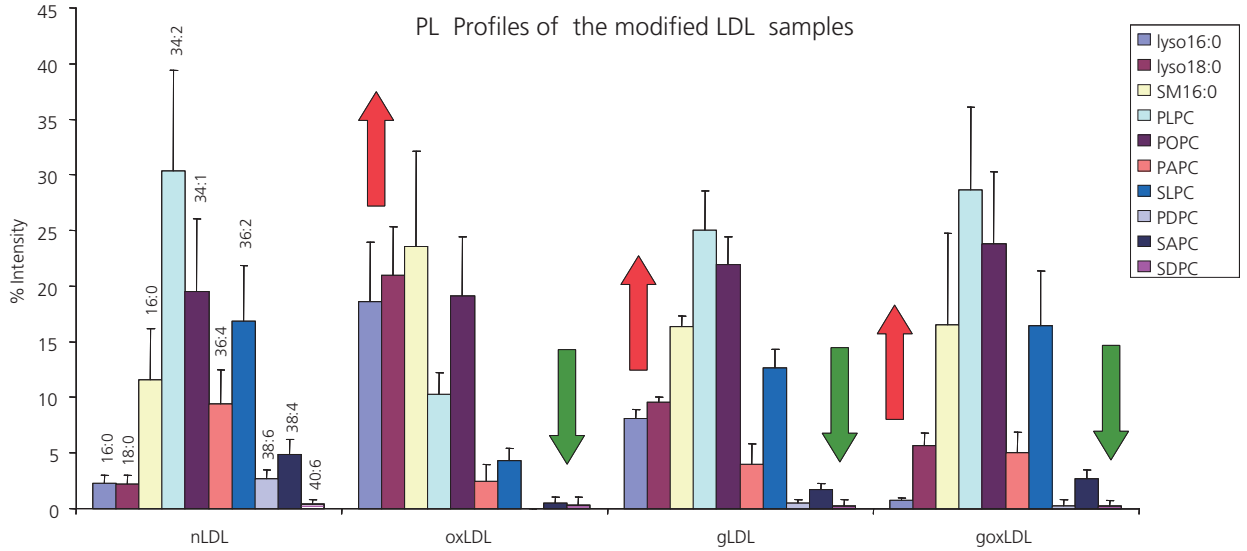

Fig. 2 Results of the MALDI Lipid Profiling of PLs isolated from different modified LDL samples.
Arrows indicate increase of LPCs (red) and decrease of PUFA-containing PCs (green) as the result of ROS-dependent degradation processes [5].

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Table 3 Component area reduction after 24Hrs treatment of oil in rat plasma

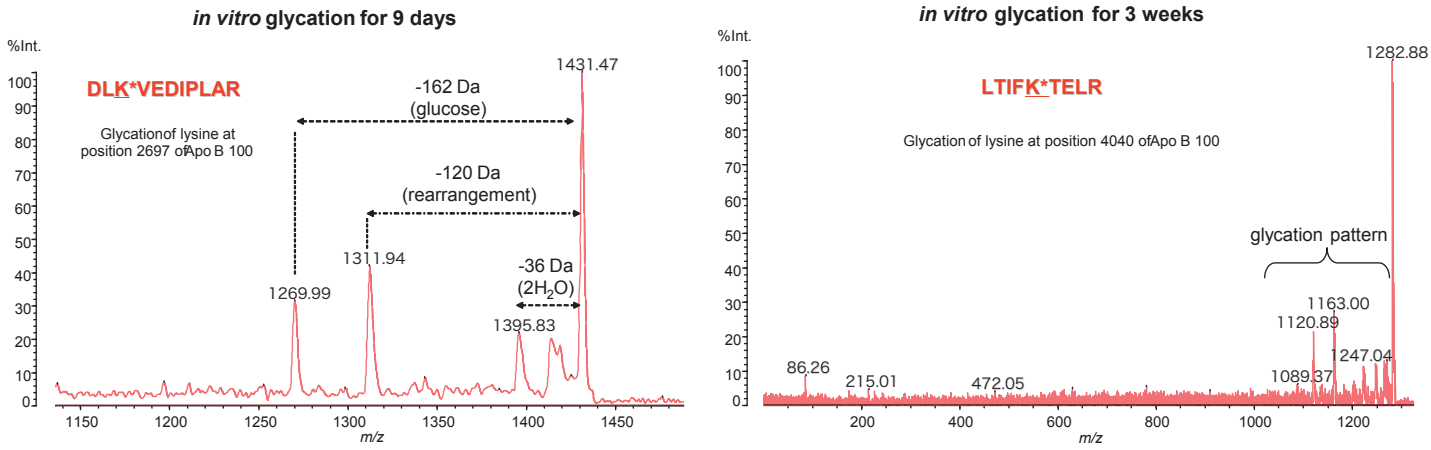

Fig. 3 Positive ion MALDI-TOF-MS/MS spectra of ApoB-100 peptides indicating glycation sites at Lysine 2697 (left) and position 4040 (right). 3 weeks of oxLDL incubation by glycose was needed to detect the modification at AA 4040.

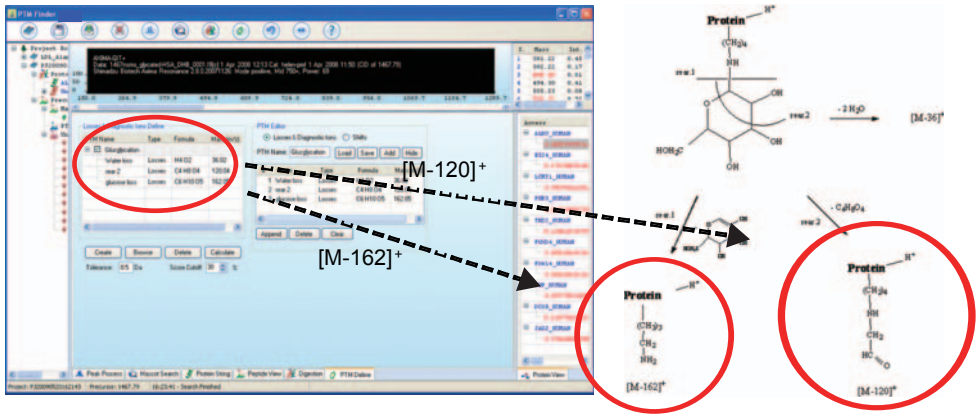

Fig. 4 PTM Finder results based on LC-MALDI-TOF-MS/MS spectra of goxLDL samples searched against a reduced protein database including ApoB-100 and human serum albumin. Known neutral losses related to sugar moieties attached to the AA backbone [7, 8] were found (red circles).

Conclusion

- Our MALDI-QIT-TOF-MS/MS based approach represents a powerful tool to study and monitor the modification process of proteins (e.g. due to the influence of ROS and/or sugars) on the constituent lipid and AA level.
- Software-assisted data analysis using the PTM Finder tool (Shimadzu, UK) allows site-specific tracking of protein modifications (e.g. glycation) within huge MALDI-MS/MS spectra datasets in a very convenient way.
- This approach shows the potential to serve as platform for monitoring of "aging processes". Such method could easily be translated in food products, pharmaceutical formulations as well as for clinical monitoring (e.g. in diabetic patients) in the future.

Literature
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