Discovery and characterization of new glucosylated metabolites: pathophysiological consequences

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Summary

Within this thesis the central stage is taken by the discovery and investigation of transglycosylation of sterols. First, investigation focuses on the development of a method to accurately detect and quantify glucosylated metabolites in biological materials. Next, the studies concentrate on the formation and occurrence of specific glucosylated metabolites, in particular glucosyl-desmosterol (GlcDesm), glucosyl-7-dehydrocholesterol (Glc7DHC) and glucosylated vitamin D_3 ($GlcD_3$).

Chapter 1 introduces lysosomes and lysosomal diseases. Specific attention is paid to the retaining lysosomal β-glucosidase named glucocerebrosidase (GBA). Inherited deficiency of this enzyme causes Gaucher disease, a relatively common lysosomal storage disorder characterized by accumulation of glucosylceramide, the simplest glycosphingolipid. The features of GBA are described with emphasis to catalysis. Besides hydrolysis, the enzyme is able at special conditions to transglucosylate, i.e., transfer a glucose from a substrate to a sterol acceptor such as cholesterol. Glucosylated cholesterol, ubiquitous in tissues, is formed in this manner. Next, two other cellular β-glucosidases, the membrane-associated nonlysosomal glucosylceramidase (GBA2) and broad-specific cytosolic β-glucosidase (GBA3) are introduced, and their known role is glycolipid metabolism is discussed. The introductory chapter is concluded by discussing the physiological relevance of transglycosylation and the possible existence of additional acceptors in transglucosylation. Attention in this respect is focused to other sterols besides cholesterol.

Chapter 2 describes the development of a (sensitive) LC-MS/MS method to quantify glucosylated metabolites in natural materials like cell and tissue extracts and plasma. Attention was paid to optimization of the extraction procedure and the LC-MS/MS detection. Use was made of synthesized ^{13}C-encoded standards for accurate and sensitive quantification of glucosylated metabolites. Attention in this respect was focused to GlcChol, GlcDesm (glucosylated desmosterol) and Glc7DHC (glucosylated-7-dehydrocholesteol). The study led to a convenient and sensitive method for quantifying GlcChol, GlcDesm and Glc7DHC in biological materials.

The glucosylated lipids were extracted by two consecutive extraction methods, Bligh and Dyer followed by butanol/water extraction before analysis by LC-MS/MS. The chapter focusses on LC-MS/MS method development, commencing with discussing linearity of the calibration curve, together with the corresponding limit of detection (LOD), limit of quantification (LOQ) and signal-to-noise ratios (S/N). This was followed by mass spectrometric analysis, fragmentation and elution spectrum of the lipids of interest. Furthermore, intra/inter assay variation and carryover of the lipids was investigated. Next, storage of the glucosylated lipids within biological material (skin, plasma, breastmilk) was checked. Impurities in the lipid standards were investigated, as well. Last, detection of the glucosylated lipids within biological samples was shown.

Chapter 3 reports on the formation, degradation and occurrence of GlcDesm, a metabolite closely resembling GlcChol that indeed undergoes comparable modification with glucose. The study revealed the *in vitro* formation and degradation of GlcDesm by GBA and GBA2 from a β-glucoside donor and desmosterol. Furthermore, in human spleen the natural presence of GlcDesm was demonstrated. Gaucher disease (GD) patients deficient in GBA, show elevated levels of GlcDesm. Given the similarity of desmosterol and cholesterol, glucosylation of the former metabolite is not unexpected. It can be concluded from the findings that GBA plays largely a role in lysosomal degradation of GlcDesm and that its synthesis is mediated by GBA2.

Chapter 4 reports on the investigation of glucosylation of 7-dehydrocholesterol (7DHC) and its metabolite vitamin D_3. The formation, degradation and natural occurrence of the glucosylated metabolites Glc7DHC and $GlcD_3$ was studied. *In vitro* formation of Glc7DHC by GBA and GBA2 was observed. Degradation was only detectable for Glc7DHC by GBA. The natural occurrence of Glc7DHC in spleen and skin was observed. In the case of $GlcD_3$ *in vitro* formation by GBA and GBA2 was also demonstrable. In addition, conversion of Glc7DHC into $GlcD_3$ by UVB irradiation was observed.

Chapter 5 considers the ability of the broad-specific β-glucosidase (GBA3) to hydrolyze and transglucosylate. Studies on GBA3-mediated formation of GlcChol as well as degradation of GlcChol, Glc7DHC, $GlcD_3$ and GlcDesm were negative. A firm conclusion that GBA3 is not active towards the studied metabolites should however be further substantiated by experiments using a purified recombinant GBA3 in larger amounts.

Chapter 6 provides a general discussion of the work described in this thesis and an outlook. It discusses additional metabolites subject to transglycosylation to be explored, focusing on cholesterol derivatives, such as oxysterols, steroid hormones and bile acids. Next, the possible biological function and the pathophysiological relevance of glucosylated metabolites and acceptor abnormality is discussed. Proposed future research concerns untargeted discovery of glycosylated metabolites, by the use of a so-called 'transbody' (a modified glucose donor) and formation and occurrence of xylosylated metabolites. In conclusion, the work presented in this thesis gives several new opportunities for research.

Addendum I is a published paper on previous work on transglucosylation of GlcChol within mammals [1]. The paper reports on the formation and degradation of GlcChol by GBA and GBA2. The presented work of this publication initiated the research of this thesis.

Addendum II contains a published paper on the ability of GBA to transxylosylate, forming xylosyl-cholesterol [2]. This work prompts to investigate the formation and degradation of xylosylated metabolites, such as xylosyl-desmosterol, xylosyl-7-dehydrocholesterol or xylosyl-vitamin D_3.

References

1. Marques, A.R., Mirzaian, M., Akiyama, H., et al., *Glucosylated cholesterol in mammalian cells and tissues: formation and degradation by multiple cellular beta-glucosidases.* J Lipid Res, 2016.
2. Boer, D.E., et al., *Human glucocerebrosidase mediates formation of xylosyl-cholesterol by β-xylosidase and transxylosidase reactions.* J Lipid Res, 2021. **62**: p. 100018.