Thesis Outline

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This thesis describes biochemical investigations on glucocerebrosidase (GBA), initially conducted at the premises of the Department of Medical Biochemistry at the Academic Medical Centre in Amsterdam and later performed at the Department of Medical Biochemistry at the Leiden Institute of Chemistry of the Leiden University. In parts of the studies use is made of novel chemical biology tools designed and synthesized at the Department of Bio-organic Synthesis at the Leiden Institute of Chemistry.

The central theme of this thesis is formed by biochemical studies on factors influencing the intralysosomal stability and survival of GBA. The steady-state GBA content of lysosomes determines the capacity of cells to degrade glucosylceramide, a reaction deficient in patients suffering from Gaucher disease (GD). The conducted investigations were aimed to increase knowledge about turnover of GBA molecules in lysosomes and to establish the feasibility of pharmacological augmentation of intralysosomal GBA degradative capacity.

Chapter 1 describes an investigation of the role of proteases in lysosomal GBA content. Building on the observation that leupeptin, a broad-specific protease inhibitor, reduces intralysosomal degradation of GBA, the nature of proteases involved in the process was analyzed in more detail. Making use of fluorescent activity-based probes directed against a number of lysosomal cysteine-proteins, the identity of cathepsins involved in proteolytic degradation of GBA was determined. An increase in functional GBA capacity in cultured lymphoblasts of GD patients with N370S enzyme was reached by inhibition of multiple cysteine cathepsins.

Chapter 2 presents an investigation of the effect of occupancy of the active site of GBA with glyco-mimetics. Permanent occupancy of the active site by covalent binding of β-glucoside configured cyclophellitols is shown to markedly stabilize GBA in its conformation and to provide protection against proteolytic degradation in cultured cells and mice.

Chapter 3 reports a new methodology to probe GBA using cyclophellitol-type activity-based probes and photoactivatable diazirine-functionalized clickable glucosylceramide (pacGlcCer). Labeling of GBA by fluorescent pacGlcCer is shown to occur with high affinity and to be prevented by prior labeling of enzyme with fluorescent β-glucopyranosyl-configured cyclophellitol. The GBA specific activity-based probe competes the binding of pacGlcCer to GBA. Cholesterol, a known lipid acceptor in transglucosylation by GBA also inhibits binding of pacGlcCer. The methodology might potentially be employed in screens for interactors with GBA.

Chapter 4 reports on the observed intralysosomal stabilization of GBA by its cellular transporter protein LIMP-2. Transient interactions of GBA with LIMP-2 in lysosomes seem to promote conformational stability of GBA and thus reduce proteolytic turnover of the enzyme. The potential implication for efficacy of high-dose enzyme replacement therapy is discussed.

Moreover, the findings suggest that treatment of AMRF patients with GBA enzyme replacement therapy is not a promising avenue.

Chapter 5 describes an investigation of LIMP-2 deficient mice with respect to abnormalities in lysosome composition, tissue GBA content and glycosphingolipid levels. Except for the absence of LIMP-2 and very marked reduction in GBA, no significant abnormalities in the proteome of isolated lysosomes of hepatocytes were identified. The tissue and cell-type specific differences in deficiency of GBA in LIMP-2 deficient mice are demonstrated, as revealed by measurement of enzymatic activity and detection of active enzyme molecules with activity-based probes. The cause for the exceptionally high residual GBA in LIMP-2 deficient white blood cells is experimentally investigated, rendering indications for re-uptake of faulty secreted GBA. An inventory of lipid abnormalities in the LIMP-2 deficient mice is also presented, revealing the successful prevention of glucosylceramide accumulation in tissues by its conversion to glucosylsphingosine.

Chapter 6 presents a review on biochemical adaptations developing during inherited primary deficiency of a glycosphingolipid-degrading lysosomal glycosidase. The active generation of glycosphingoid bases from accumulating glycosphingolipids by lysosomal acid ceramidase is described and the potential pathophysiological consequences of excessive glycosphingoid bases are discussed. In addition, the increased metabolism of glucosylceramide by the cytosolic β-glucosidase GBA2 during deficiency of lysosomal GBA activity is reviewed and the potential consequences of the related excessive formation of ceramide and glucosylated metabolites in the cytosol are considered.

Chapter 7 deals with the catalytic versatility of GBA. Next to hydrolyzing β-glucosides, the enzyme also cleaves β-xylosides, although with lower affinity. Moreover, GBA is able to act as a xylosyltransferase. Incubation of enzyme with 4-methylumbelliferyl-β-xyloside (4MU-β-Xyl) leads to formation of xyloslated cholesterol (XylChol) and dixylosylated sterol (Xyl_2Chol). Incubation of cultured cells with 4MU-β-Xyl also causes formation of XylChol and Xyl_2Chol, a reaction promoted by U18666A induced lysosomal cholesterol accumulation and being prevented by inactivation of GBA with conduritol B-epoxide.

The ***Addendum*** describes the multiplex quantitation of a series of glycosphingoid bases in plasma samples using ^{13}C encoded internal standards. It is further demonstrated that glycosphingolipids in the same sample can be quantified following microwave-assisted de-acylation to the corresponding bases.

The ***Discussion*** concludes the thesis and concerns the implications of the findings in view of recent literature and insights. Envisioned future directions of research are indicated.

The ***Summary*** presents an overview of the various studies presented in this thesis.