Starch is the most abundant storage carbohydrate in cereal grains. It is composed primarily of amyllopectin, a polymer of glucose in which α-1,4-linked glucan chains are branched with α-1,6-bonds. Enzymatic degradation of starch in germinating barley seeds involves an initial solubilization, mainly by α-amylase, followed by hydrolysis of the resulting dextrins to oligosaccharides and glucose by the concerted action of α- and β-amylase, limit dextrinase (LD), and α-glucosidase. Only LD is able to hydrolyze α-1,6-linkages in limit dextrans. Since LD is the sole debranching enzyme in the germinating seed, it has a key role in malting and brewing. But an endogenous inhibitor, limit dextrinase inhibitor (LDI), is present in barley seeds. It specifically inhibits LD, and thereby suppresses the degradation of branched limit dextrans to fermentable sugars. The knowledge about this enzyme:inhibitor pair and inhibitors of debranching enzymes is sparse.

During the last decades knowledge about LD has improved, recently with the crystal structures of LD in complex with the competitive inhibitors α- or β-cyclodextrin. But deeper insight into the substrate specificity determinants at molecular level is still sparse. Here I present crystal structures of LD, and LD in complex with 6α-maltotriosyl-maltotriose, i.e. a limit dextrin, or two maltotriose molecules. The branched ligand is in contact with LD via interactions between all six glucose units and amino acid residues of LD. The active site cleft of LD can accommodate longer side chains, while the active site topology at both + and – subsites could cause steric hindrances. Met440 was proposed to be such a hindrance near subsite –3 based on structure comparisons between one of the first published structures of LD and a related enzyme from Klebsiella pneumoniae. LD-related enzymes with higher activity towards amyllopectin as compared with LD in general have a cysteine or a glycine, at the equivalent position, thus an LD-M440G variant was produced. The kinetic constants of wild type LD on amyllopectin were determined to be $K_m = 6.9 \pm 1.0$ mg/ml and $k_{cat} = 15.6 \pm 1.2$ s$^{-1}$, resulting in a catalytic efficiency ($k_{cat}/K_m$) of 2.3 ml(mg s)$^{-1}$. This is more than 200-fold lower than the catalytic efficiency on pullulan. The LD-M440G variant had an even 2-fold lower $k_{cat}/K_m$ (0.9 ml(mg s)$^{-1}$) on amyllopectin. Based on this it is concluded that Met440 does not account for the low activity of LD on polymeric substrates. The structure of LD in complex with a branched substrate provides new possibilities for structural comparisons with other debranching enzymes. Active site topology elements of LD, like Phe553, were identified as possible substrate specificity determinants based on these comparisons.

In the second part of the thesis I present the crystal structure of the complex between LD and LDI determined to 2.7 Å. The structure revealed a novel mode of inhibition distinctly different from that of the interaction between LDI-like inhibitors and α-1,4-acting enzymes (α-amylases) from the same glycoside hydrolase family as LD (GH13), which involves the opposite face of LDI. Taking advantage of this LD:LDI structure a number of LD and LDI variants were constructed to elucidate hot spots of the interaction as well as to engineer LD to be less sensitive towards LDI. Mutations of residues in a hydrophobic cluster of LDI caused the most dramatic decreases in binding affinity. The LDI-L41G-V42D variant had close to $5 \times 10^5$-fold reduced affinity. The complex formation was independent of ionic strength, which was confirmed by the minor importance of two LDI arginines, Arg34 and Arg38, for the binding affinity. Arg38 thus interacted with two of the three catalytic site residues of LD. The LD:LDI structure also guided a successful LD mutation, D730R, reducing the sensitivity towards LDI by 180-fold without changing the LD kinetics on pullulan.