## **SUMMARY**

## The α-glucan phosphorylases of the glycosyltransferase family are important enzymes of carbohydrate metabolism in prokaryotes and eukaryotes. Glycogen and starch serve as energy stores in animals and plants, respectively.

The majority of glycogen is stored in the liver and skeletal muscle. A main function of glycogen is to maintain a physiological blood glucose concentration, but only liver glycogen directly contributes to release of glucose into the blood.

Glycogen phosphorylase catalyzes the first step in the intracellular degradation of glycogen to yield glucose 1-phosphate (G1P). Apart from the enzyme active site several other peripheral binding sites have been reported for GP, the allosteric, new allosteric, inhibitor, quercetin, and glycogen storage site. These sites provide a range of options for the design of structurally diverse inhibitors with therapeutic potential as antihyperglycemics for Type 2 Diabetes patients.

In this Thesis a series of compounds designed to bind to the peripheral GP binding sites were studied by kinetics and X-ray crystallography. The inhibitory potency of a collection of 75 polyphenol compounds (flavonoids and anthocyanidins) was studied. The most potent inhibitor was found to be baicalein (*K*i = 5.3 μΜ) which binds at the inhibitor site. Pelargonidin (an anthocyanidin), was found bound at the quercetin binding site of GP displaying a *K*i value of 31.1 μΜ for hlGPa. **G02** was the most potent inhibitor among those that bind at the allosteric site of the enzyme (*K*i = 5.8 µM). The inhibitory potency of another collection of 10 polyaromatic compounds was also evaluated against GP. The most potent inhibitor for hlGPa was **PA7** (Ki = 7.87 μΜ) and it was found bound at the new allosteric site.

Starch is an insoluble polymer of glucose residues produced by most higher plant species and is a major storage product of many of the seeds and storage organs produced agriculturally and used for human consumption. All higher plant starches are synthesized inside plastids. Their phosphorolytic degradation is catalyzed by starch phosphorylases (SP) and to date little is known about plastid starch phosphorylases (Pho1). We present here a biochemical analysis of *st*Pho1 together with the crystal structure of *st*Pho1ΔL78, a form of *st*Pho1 which is composed by two segments generated by proteolytic degradation of an approximately 65 residue long peptide (L78) located in the middle of the *st*Pho1 primary structure. *st*Pho1ΔL78 is 1.5 times more active than the non-proteolyzed enzyme in solution and shows stronger specificity than *st*Pho1 for glycogen, α-D-glucose, caffeine, and β-cyclodextrin. The crystal structure of *st*Pho1ΔL78 has been resolved at 2.2 Å resolution and revealed similarities and differences to the mammalian enzymes. The structural fold is conserved as is the active site, and the indole binding site while others such as the inhibitor, the glycogen storage, the quercetin, and the allosteric are not. The binding of α-D-glucose, caffeine, and β-cyclodextrin to *st*Pho1 has been studied by X-ray crystallography and revealed significant differences from the mammalian phosphorylases. As *st*Pho1 is capable of catalyzing both starch synthesis and degradation our crystal structures suggested that the direction of *st*Pho1 activity is regulated through proteolytic degradation of the L78 peptide. This is the first time that a plant phosphorylase has been biochemically and structurally characterized in detail and might be the starting point for further studies in plant phosphorylases.