CHAPTER I

INTRODUCTION

Starch is a basic constituent of the human and animal diet. It is an important carbohydrate considered as one of the primary energy source for plants and a very important raw material for industrial processes.

In many different plant species it has been demonstrated that ADP-glucose pyrophosphorylase (AGPase) (EC 2.7.7.27) is one of the major enzyme for starch biosynthesis. The overall crop yield potential is greatly influenced by the enzyme which modulates the photosynthetic efficiency in source tissues as well as determines the level of storage starch in sink tissues [Salamone et al., 2000]. Combined participation of AGPase, starch synthase and branching enzyme are solely responsible for biosynthesis of starch in plants [Martin and Smith, 1995; Preiss, 1991]. In starch biosynthesis, AGPase is the first regulatory allosteric enzyme which converts ATP and glucose-1-phosphate (Glc1P) to adenosine-5'-diphosphoglucose (ADPGlc) and inorganic pyrophosphate (PPi) [Preiss, 1984; Slattery et al., 2000; Ballicora et al., 2004; Kleczkowski, 2000; Tiessen et al., 2002].

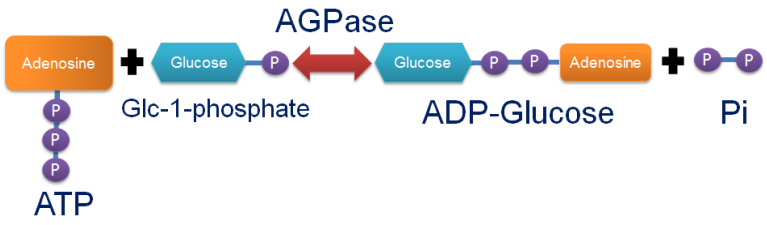

Figure 1: Conversion of ATP and glucose-1-phosphate (Glc1P) to adenosine-5'-diphosphoglucose (ADPGlc) and inorganic pyrophosphate (PPi) in presence of ADP-glucose pyrophosphorylase (AGPase).

Mutant analysis and transgenic plants provide strong evidences of the allosteric properties of AGPase in controlling the rate of starch biosynthesis in higher plants [Tsai and Nelson, 1996; Hannah and Nelson, 1976; Linn et al., 1988; Muller-Rober et al., 1992; Stark et al., 1992]. In most cases, the regulation of AGPase depends on the ratio of 3-phosphoglyceric acid (3-PGA), the product of CO_2 fixation and inorganic phosphate (Pi) showing a direct correlation between the concentration of 3-PGA and starch accumulation and an inverse correlation between Pi concentration and the starch content [Iglesias et al., 1993]. Although the overall kinetic mechanism of AGPase appears to be similar in bacteria and higher plants, their quaternary structures differ from each other [Preiss, 1991]. Bacterial AGPases are composed of four identical subunits (α) to form a α4 homotetramer whereas plant AGPases are heterotetramer of two different yet evolutionarily related subunits containing a pair of identical small (SS or α) and identical large (LS or β) subunits to form α2β2 heterotetramer [Iglesias et al., 1993; Haugen et al., 1976; Okita et al., 1990; Ballicora et al., 2003]. The two subunits vary in their molecular weight, genetic origin and are encoded by two different genes [Okita et al., 1990; Smith-White and Preiss, 1992]. Primary sequence analysis of LS and SS of AGPase has shown that SS have considerable sequence homology among species, while the LS are less homologous [Smith- White and Preiss, 1992]. Despite the sequence divergence between SS and LS, the subunits are paralogs, suggesting that they were encoded by a common ancestral gene [Smith- White and Preiss, 1992]. Smith-White and Preiss (1992) suggested that the SS were under more selective constraint than the LS which was evident by the fact that the cyanobacterial AGPases are more identical with the SS [Greene et al., 1996]. In several plants, LS are present in multifamilies [Villand et al., 1993; La Cognata et al.,

1995; Villand et al., 1992a; Chen et al., 1998] and highly tissue specific in expression pattern, restricted to either leaf, root, or endosperm in barley, potato, and tomato [La Cognata et al., 1995; Chen et al., 1998; Villand et al., 1992 a,b]. In contrast, SS are not always found in multifamilies [Weber et al., 1995; Giroux and Hannah, 1994; Prioul et al., 1994].

Both the subunits have different roles in enzyme functionality. Several researchers have reported that SS of AGPase have both catalytic and regulatory functions whereas LS has only regulatory function [Ball and Preiss, 1994; Ballicora et. al., 1995; Greene et. al., 1996; Ballicora et. al., 1998; Laughlin et. al., 1998; Kavakli et. al., 2001]. When expressed in the absence of the LS, the *Solanum tuberosum* SS is capable of forming a catalytically active homotetrameric AGPase, whereas LS is incompetent of forming an oligomeric structure with catalytic activities [Salamone et al., 2000; Iglesias et al., 1993; Ballicora et. al., 1995; Salamone et al., 2002]. Although *Solanum tuberosum* SS forms a homotetramer, it is allosterically defective requiring more than 15-fold greater levels of 3-PGA for its activation in comparison to the heterotetrameric enzyme. Moreover, Kavakli and co-workers (2001) and Hwang and co-workers (2006, 2008) suggested that the LS may bind to substrate ATP as well as Glc1P and may allow the LS to interact in tandem with the catalytic SS influencing the net catalysis. In addition, specific regions of both the LS and the SS were found to be important for enzyme stability and subunit association [Salamone et al., 2000]. Mutations affecting catalytic and allosteric properties of AGPase map to both subunits [Cross et al., 2004, Hwang et al., 2005]. Study on chimeric maize/potato SS reflects a polymorphic motif of 55-amino acid region between the residues 322-376 which plays a critical role during the interaction with LS and

contributes to the overall stability of the enzyme [Cross et al., 2005]. All these reports suggest that both the subunits are of equal importance for the net catalysis and allosteric regulation of the enzyme.

The thermal stability of AGPase is also an important characteristic because exposure to high temperatures during plant growth causes reduced grain yield in many internationally important cereal crops, including maize, wheat, and rice [Singletary et al., 1994]. Investigations by others have shown that AGPase is one of the enzymes most affected by elevated temperature [Singletary et al., 1994]. Most plant AGPases, for example, the potato tuber enzyme [Sowokinos and Preiss, 1982; Okita et al., 1990] are heat stable, whereas the cytosolic endosperm isoforms are quite labile. Incubating the maize endosperm AGPase at 57°C for 5 min destroys 96% of the activity [Hannah et al., 1980], whereas the potato tuber enzyme is fully stable at 70°C. Expression of a heat-stable AGPase in the seed of wheat [Smidansky et al., 2002], rice [Smidansky et al., 2003], and maize [Giroux et al., 1996] increases grain yield.

Transgenic approaches have shown that AGPases harboring alterations in allosteric properties as well as enhanced thermal stabilities can enhance starch synthesis in agriculturally important tissues and organs such as the potato tuber and seeds of maize, wheat, and rice. Metabolites that activate or inhibit AGPase also stabilize the enzyme to thermal inactivation [Zhou and Cheng, 2005; Boehlein et al., 2008]. This enzyme plays a key role in the modulation of photosynthetic efficiency in source tissues and also determines the level of storage starch in sink tissues, thus influencing overall crop yield potential as well as quality. Fruit quality in tomato is largely a function of the soluble solid content of the fruit, and there is a direct relationship between starch levels early in

fruit development and the soluble solids content at maturity [Davies and Cocking, 1965; Dinar and Stevens, 1981; Schaffer and Petreikov, 1997]. Increased starch biosynthesis in fruits may also lead to increased assimilate partitioning into fruit, and a decrease in non-edible biomass, reducing waste processing needs. Thus, AGPase appears to be an attractive target for increasing starch synthesis in plants.

The three dimensional (3D) structure of a protein is essential for determining its function. Due to the difficulty of obtaining AGPase in stable form neither the LS nor the heterotetrameric AGPase ($\alpha 2\beta 2$) atomic resolution structure from plant species have been solved yet. In 2005, Jin and co-workers reported the first atomic resolution structure of SS of AGPase from *Solanum tuberosum*. The crystal structure of SS was found in a homotetrameric form. Since then not a single crystallographic structure of AGPase has been reported. Although AGPases offer an attractive tool for engineering crop plants to enhance the yield potential of starch content, understanding of structure-function relationships and the unique substrate specificity of AGPase have remained elusive.

Comparative modeling coupled with docking study has been immensely used for understanding the structure-function relationship, mode of enzyme substrate interaction and the residue involved in interaction without requiring further biochemical or immunological data [Witz et. al., 1993; Kamaraj and Purohit, 2013; Xu et. al., 2013; Lakshmanan et. al., 2013; Kolodny and Kosloff, 2013; Wallrapp et. al., 2013; Le Gac et. al., 2013; Chaturvedi et. al., 2013; Kashyap, 2013; Sarma et. al., 2012; Rai and Rieder, 2012; Ganguly and Prasad, 2012; Thorsell et. al., 2011; Manning et. al., 2010; Chen and Pellequer, 2004; Peterson and Graham, 1998]. Despite recent advances in the identification of the regulatory and catalytic residues in the higher plant AGPase, detail

mechanism of enzyme function and regulation is not fully understood. Therefore in the present study the following objectives were set to highlight the important structural aspects of AGPase to have more information about the roles of the AGPase in enzyme function in selected crop species.

OBJECTIVES

- Molecular modeling of both small and large subunit of AGPase using available crystal structures.
- Hetero-tetrameric assembly of AGPase subunits through docking.
- Identification and characterization of critical amino acid residues or particular motif involved in allosteric regulation, enzyme activity and thermostability of AGPase.
- To elucidate the evolutionary relationship, construct the phylogenetic tree of both small and large subunits of AGPase.
- *In silico* site-directed mutagenesis of specific amino acid residues or particular motif responsible for enzyme activity.