Biotechnology and Molecular Biology Review Vol. 7(3), pp. 69-83, September 2012
Available online at http://www.academicjournals.org/BMBR
DOI: 10.5897/BMBR12.004
ISSN 1538-2273 ©2012 Academic Journals

Review

Starch phosphorylase: Biochemical and biotechnological perspectives

Rachana Tiwari and Anil Kumar*

School of Biotechnology, Devi Ahilya University, Khandwa Rd., Indore-452001, India.

Accepted 5 June, 2012

A dynamic mediatory role between starch synthesis and degradation has been ascribed to starch phosphorylase. However, plant starch phosphorylase is largely considered to be involved in phosphorolytic degradation of starch. It reversibly catalyzes the transfer of glucosyl units from glucose-1-phosphate to the non-reducing end of glucan chain with the release of inorganic phosphate. It is widely distributed in plant kingdom. Enzyme multiplicity is also common in starch phosphorylase and different multiple forms have been predicted to have different roles in starch metabolism. Here, various biochemical properties have been reviewed. Its regulation by aromatic amino acids has also been discussed. Importance of plastidial and cytoplasmic starch phosphorylase has also been discussed. Various biotechnological aspects have been discussed. Its exploitation in production of glucose-1-phosphate, a cytostatic compound has been discussed in the present review.

Key words: Starch phosphorylase, glucose-1-phosphate, amylase, biosensor, aromatic amino acids.

INTRODUCTION

In plant carbohydrate metabolism, a dynamic mediatory role between starch synthesis and degradation has been ascribed to starch phosphorylase (Schneider et al., 1981). Besides, other glycosyl transferring phosphorrylases are sucrose phosphorylase (EC. 2.4.1.7); maltose phosphorylase (EC. 2.4.1.8); cellobiose phosphorylase (EC. 2.4.1.20.); 1,3-β-oligoglucan phosphorylase (EC. 2.4.1.30); laminaribiose phosphorylase (EC. 2.4.1.31); cellodextrin phosphorylase (EC. 2.4.1.49); and α-α-trehalose phosphorylase (EC. 2.4.1.64). The catalytic activity of starch phosphorylase is restricted to the non–reducing end of starch. Starch phosphorylase is a form similar to glycogen phosphorylase, except that it acts upon starch instead of glycogen. The plant α- glucan phosphorylase commonly called as starch phosphorylase (EC 2.4.1.1) is largely known for phosphorolytic degradation of starch. It catalyzes the reversible conversion of α-1,4-glucan and inorganic phosphate into glucose-1-phosphate, and plays an important role in starch metabolism.

$$(1,4\text{-}\alpha\text{-D-glucosyl})_n + \text{orthophosphate } (P_i) \underset{\text{starch synthesis}}{\overset{\text{starch degradation}}{\rightleftharpoons}} (1,4\text{-}\alpha\text{-D-glucosyl})_{n-1} + \alpha\text{-D-glucose-1-phosphate}$$

It has recently been reported that reversible phos-phorylation of starch granule is essential for its subsequent degradation (Fettke et al., 2007). Within starch granules, glucan chains of amylopectin fraction form double helices and get packed to form crystalline lamellae and double helices at the granule surface and get disrupted by the

*Corresponding author. E-mail: ak_sbt@yahoo.com. Tel: 91-731-2470372.

Abbreviations: Pi, orthophosphate; **mRNA,** messenger ribonucleic acid; **Pho1,** phosphorylase 1; **Pho2,** phosphorylase 2; **KDa,** kilodalton; **Da,** Dalton; **P1,** phosphorylase 1**; P2,** phosphorylase 2; **P3,** phosphorylase 3; **L-form,** light form; **H-form,** heavy form; **ADPglucose,** adenosine diphosphate glucose; **L78,** 78 amino acids long segment; **Km,** Michaelis constant; **AMP,** adenosine monophosphate; **cAMP,** 3',5'-cyclic adenosine monophosphate; **ATP,** adenosine triphosphate; **UDPglucose,** uridine diphosphate glucose; **GDPglucose,** guanidine diphosphate glucose; **NADP$^+$,** nicotinamide adenine dinucleotide; **NADPH,** reduced nicotinamide adenine dinucleotide; **GT,** glycosyl transferase; **PEST-region,** proline, glutamic acid, serine, threonine enriched region.

addition of phosphate groups and individual glucosyl residues. Phosphate addition is mediated by two enzymes, glucan water di kinase (EC. 2.7.9.4) and phosphoglucan water di kinase (EC. 2.7.9.5), respectively. Evidence for the importance of starch phosphorylation *in vivo* comes from the analysis of these enzymes of mutant plants. These plants exhibit reduced growth and accumulate nearly phosphate-free starch to a high level (Zeeman et al., 2007). The glucan water dikinase phosphorylates starch at C6 whereas phosphoglucan water dikinase phosphorylates starch at C3 position has been predicted by molecular modeling (Kossmann and Lloyd, 2000; Blennow et al., 2002; Wischmann et al, 1999; Rathore et al., 2009).

Distribution

Starch phosphorylase is widely distributed in plant kingdom (Fukui, 1982; Steup, 1988; Kumar, 1989). Starch phosphorylase has been described in many plants viz. potato tuber (Kamogawa et al., 1968; Tsay and Kuo, 1980; Schneider et al., 1981; Mohabir and John, 1988; Brisson et al., 1989); sweet potato tuber (Ariki and Fukui, 1975; Chang et al., 1987; Chern et al., 1990; Chiang et al., 1991; Lin et al., 1991); tapioca tuber (Vishwanathan and Krishnan, 1965, 1966; Kumar and Sanwal, 1984), developing maize seeds (Tsai and Nelson, 1969, Tsai et al., 1970); maize kernels (Ozbun et al.., 1973), maize endosperm (Tsai and Nelson, 1968; Chao and Seandalios, 1969); developing barley seeds (Batra and Mehta, 1981); pea seeds (Matheson and Richardson, 1976, 1978; Yang and Steup, 1990); germinating pea seeds (Juliano and Varner, 1969); mung bean and broad bean (Richardson and Matheson, 1977); germinating wheat seeds (Abbott and Matheson, 1972); spinach leaves (Preiss et al., 1980); *Zea mays* leaves (John et al., 1973a; Mateyka and Schnarren-berger, 1984, 1988; Spilatro and Preiss, 1987); *Vitis vinifera* leaves (John et al., 1973b; Downton and Hawker, 1973); loranthus leaves (Khanna et al., 1971); tobacco leaves (Vishwanathan and Shrivastava, 1964); *Aspergillus orisous* (Vishwanathan and Shrivastava, 1964); tapioca leaves (Kumar and Sanwal., 1982b); pea leaves (Steup and Latzko, 1979; Conrads et al., 1986; Yang and Steup, 1990); young banana leaves (Kumar and Sanwal, 1982a), mature banana leaves (Kumar and Sanwal, 1981a, 1983a, b); cabbage leaves (Kumar, 1984; Garg and Kumar, 2008); Indian millet (Garg and Kumar, 2007); *Arabidopsis thaliana* (Caspar et al., 1991); yan (Hamdon and Diapoh, 1991); *Vicia faba* leaves (Ghiena et al., 1993); *Saffron crocus* (Chrungoo and Farooq, 1993), and in developing soyabean seeds (Michiko et al., 1991). Singh et al. (1967) reported the presence of starch phosphorylase activity in the leaves of *Cajanas cajan*, *Coccinia indica, Impatiens balsamina, Lantana camara, Luffa acutangula, Momordica charantia, Murrava koeniggi, Nerium indicum, Ocimum americanum, Pitheullobium dulce, Santalum album and Tecoma stans.* John et al. (1973b) showed the presence of starch phosphorylase in *Vitis vinifera* berries. Starch phosphorylase has also been reported in banana fruits (Singh and Sanwal, 1975), blue green algae (Fredrick, 1967), *Chlorella vulgaris* (Nakamura and Imamura, 1983; Miyachi and Miyachi, 1987); red sea weed (Yu and Pedersen, 1991, 1991a) red algae (Yu, 1992) and *Escherichia coli* (Yu et al., 1988).

Enzyme multiplicity

Multiple forms of starch phosphorylase have been demonstrated in a number of tissues. The abundance of multiplicity has been suggested as a biochemical response to the heterogeneity of starch molecules and starch granules structure (Beck and Ziegler, 1989). It can result from transcriptional level due to different genes, post transcriptional level, due to splicing of mRNA and post transcriptional level due to glycosylation, proteolytic modifications and aggregation etc (Yu and Pedersen, 1991a). The significance of multiple forms of starch phosphorylase in the regulation of starch metabolism has been suggested. In general, the multiplicity range of starch phosphorylase varies from 2 to 5 (Nighojkar and Kumar, 1997).

Multiple forms of starch phosphorylase have been reported in blue green algae (Fredrick, 1967); spinach (Okita et al., 1979; Steup and Latzko, 1979; Steup et al., 1980, 1980a; Preiss et al., 1980; Shimomura et al., 1982; Schachtele and Steup, 1986), *Vicia faba* leaves (Gerbrandy and Verleur, 1971), *Hordeum distichum* (Baxter and Duffus, 1973), maize leaves (Mateyka and Schnarrenberger, 1984; Spilatro and Preiss, 1987; Mateyka and Schnarrenberger, 1988), pea leaves (Richardson and Matheson, 1977; Levi and Preiss, 1978; Steup and Latzko, 1979; Conrads et al., 1986), red sea weeds (Yu and Pederson, 1991, 1991a), banana leaves (Kumar and Sanwal, 1977), marigold, turnip, cabbage, brinjal, arhardal (*Cajanus*), potato leaves (Kumar and Sanwal, 1982c), potato tuber (Slabnick and Frydman, 1970; Gerbrandy and Verleur, 1971; Gerbrandy and Doorgeest, 1972; Shivaram, 1976), loranthus leaves (Khanna et al., 1971), *Allium cepa* and *Phaseolus vulgaris* (Gerbrandy and Verleur, 1971); sweet corn (Lee, 1972; Lee and Broun, 1973), young and mature banana fruits (Singh and Sanwal, 1975) germinating pea seeds (Matheson and Richardson 1976; Van berkel et al., 1991; Myers and Matheson, 1991), corms of saffron crocus (Chrungoo and Farooq, 1993).

Steup and Latzko (1979) reported two multiple forms in spinach leaves named as Pho1 and Pho2. The Pho1 has low affinity for glycogen and is located in the chloroplast, and Pho2 has high affinity for glycogen and is located in the cytosol (Steup and Latzko, 1979). The subunit molecular weight of Pho2 is found to be about 92 KDa

whereas native molecular weight was 194 KDa. The subunit molecular weight of Pho1 is found to be about 104 KDa whereas native molecular weight is reported to be 204 KDa (Preiss et al., 1980).

Three multiple forms of starch phosphorylase are reported in wheat (*Triticum aestivum* L. var. *star*) as resolved by non-denaturing polyacrylamide gel affinity electrophoresis and named as P1, P2 and P3. Compartment analysis of young leaf tissues showed that P3 is plastidic whereas P1 and P2 are cytosolic (Schupp and Zeigler, 2004).

The multiplicity of starch phosphorylase has been shown in different developmental stages with respect to starch synthesis or degradation in maize endosperm (Tsai and Nelson, 1968, 1969), banana fruit pulps (Singh and Sanwal, 1975), growing as well as sprouting tubers and other plant propagules (Gerbrandy and Verleur, 1971).

This multiplicity does not coincide with any functional redundancies, as each form appears to have acquired a distinct and conserved role in starch metabolism (Ball and Morell, 2003). The multiple forms have been readily differentiated by their kinetic behavior or compartmentation. In potato (*Solenum tuberosum*) tubers and leaves, two forms L- and H-types, located in plastids and cytosol have been described corresponding to the low and high affinity for glucans (Mori et al., 1993a). In banana plant, two forms have been isolated in the leaves of *Musa paradisiaca* (type-A and -B) and fruits of *Musa acuminata* (II and I) (Kumar and Sanwal, 1977; Kumar and Sanwal, 1982a; da Mota et al., 2002). Similar pairs of phosphorylases have been found in *Arabidopsis* (AtPHS1 and AtPHS2) (Zeeman et al., 2004b), sweet potato (*Ipomoea batatas)* (Chen et al., 2002) and other higher plants. In general, most of the higher plants possess two types of starch phosphorylases, termed as Pho1 or L-form (plastidic) and Pho2 or H-form (cytosolic). The plastidial L-form has monomeric size of approximately 105,000 Da and has low affinity to branched glucans, glycogen and starch. On the other hand, the H-form has monomeric size of approximately 90,000 Da and has very high affinity to linear and branched glucans. Recent studies indicated that H-form also acts on heteroglycans (Fettke et al., 2004, 2005a, b). A great deal of confusion exists with regard to the nomenclature of these enzymes as in many cases it is not clear if they are isozyme (genetically different).

Importance of starch phosphorylase

Starch and sucrose are the primary products of photosynthesis in the leaves of plants. Starch exhibits major plant storage carbohydrate which gives energy during the times of heterotrophic growth. Starch metabolism has been studied extensively leading to a good knowledge of numerous enzymes involved. On the contrary, understanding of the regulation of starch metabolism is fragmentary (Kötting et al., 2010).

The physiological substrate and biological accurate functioning of cytoplasmic phosphorylase are not yet known in higher plants even after working nearly seventy years. However, there is some information available about the regulation of plant phosphorylase. It is well known that starch phosphorylase is a starch degradative enzyme. Starch biosynthesis takes place by ADPglucose pyrophosphorylase and starch synthase. However, Steup (1988) has shown the role of starch phosphorylase in photosynthetic carbon metabolism.

There are a number of reports for the homogeneous purification and biochemical characterization of starch phosphorylase (Kumar, 1989; Nighojkar and Kumar, 1997). The occurrence of multiple forms and their distinct intra-cellular localization are characteristics of starch phosphorylase (Nighojkar and Kumar, 1997). The heterogeneity of enzymes catalyzing apparently the same reaction is one of the characteristic features of almost all the starch degrading enzyme (Smith et al., 2003; Li et al., 2007; Rathore et al., 2009). Some multiple forms may favor starch biosynthesis over degradation (Gerbrandy and Doorgeest, 1972). Some multiple forms of starch phosphorylase favor starch biosynthesis and the starch thus synthesized may act as a primer for ADP glucose starch synthase (Frydman and Slabnik, 1973).

There are evidences that starch phosphorylase probably contributes to starch synthesis as expression and activity of plastidial starch phosphorylase that strongly correlates with starch synthesis (Brisson et al., 1989; Sonnewald et al., 1995; Yu et al., 2001; Tetlow et al., 2004b; Hannah and James, 2008). Starch phosphorylase in potato (Albrecht et al., 2001) and pho-A and pho-B in *Chlamydomonas reinhardtii* (Dauvillee, 2006) have been shown to involve in starch synthesis. Satoh et al. (2008) have shown that starch phosphorylase plays a crucial role in starch biosynthesis in rice endosperm, and appreciably affects the starch structure. According to the speculative glucan trimming model (Ball et al., 1996; Ball and Morell, 2003), during discontinuous synthesis of starch granules, the short glucan chain released from pre-amylopectin by the action of debranching enzymes are converted to longer glucan chains. The long glucan chains then act as substrate for starch phosphorylase. Recently, a new hypothesis has emerged that proposes multi-protein complex machinery for starch metabolism. It has been shown that phosphorylation of starch phosphorylase and branching enzymes promotes selective protein-protein interaction leading to multi-protein complex and assembly is essential for starch synthesis and degradation (Tetlow et al., 2004a, 2004b; Hennen-Bierwegan et al., 2009).

Chang et al. (1987) reported that the low molecular weight forms of starch phosphorylase stored in roots of sweet potato originate by proteolysis from high molecular weight phosphorylases present in the growing and

developing tubers. The accumulation of starch takes place both in the chloroplasts and amyloplasts whereas starch phosphorylase is found in the cytosol and plastids (Schneider et al., 1981; Kumar, 1989).

Importance of plastidial starch phosphorylase in plants

The precise *in vivo* role of plastidic phosphorylases is not yet clear. On the basis of many indirect evidences, it is concluded that starch phosphorylase is primarily involved in phosphorolytic starch degradation. However, the enzyme cannot directly act on intact starch granules. The low affinity of starch phosphorylases for large and branched substrates supports its inability to act on intact starch granules (Zeeman et al., 2004a). The phosphorolytic action of starch phosphorylase on starch is expected across α-1,6-branch point, beyond which the enzyme is unable to act. The starch granules are degraded into branched and linear polyglucans. The branched polymers are reduced to linear glucans by debranching enzymes such as isoamylase (E.C. 3.2.1.68) and β-limit dextrinase (E.C. 3.2.1.142) which specifically cleave α-1, 6-glycosidic bond.

The linear glucans are further degraded by β-amylase and starch phosphorylase into neutral sugar (Smith et al., 2005). However, as the major pathway of starch degradation comes from hydrolysis, β-amylase is considered to be an important enzyme in starch degradation. Therefore, absence of plastidic phosphorylase has no effect on starch degradation. Rather, it has been observed that starch phosphorylase plays an important role in stress tolerance pathway. The exposure to transient dehydration stress leads to the activation of starch phosphorylase that in turn triggers starch degradation. It has been argued that during stress, phosphorolytic starch degradation serves a swift response to ease stress by routing the substrate into oxidative hexose monophosphate pathway (Zeeman et al., 2004b; Lloyd et al., 2005; Rathore et al., 2009).

There are some evidences to show that plastidial starch phosphorylase probably contributes in starch synthesis since expression and activity of plastidial starch phosphorylase favors its role in starch synthesis (Brisson et al., 1989; Sonnewald et al., 1995; Yu et al., 2001; Tetlow et al., 2004b; Hannah and James, 2008; Rathore et al., 2009).

Importance of cytosolic phosphorylase in plants

The physiological substrate and biological functions of cytosolic starch phosphorylase are yet not exactly clear. It is very unlikely that cytosolic starch phosphorylase acts on intact starch granules which are synthesized inside the chloroplast since chloroplastic membrane is impermeable to the starch as well as the enzyme. The mutant studies with potato showed that cytosolic starch phosphorylase had no effect on starch degradation. Studies using antisense RNA for cytosolic phosphorylase indicated that it had effect on sprout growth and flowering (Duwenig et al., 1997; Rathore et al., 2009). The cytosolic phosphorylase (Pho2) lacks L78 insert, which sterically hinders the substrate binding and therefore is more effective in degradation of branched large glucans and therefore may act on intact starch granules (Steup et al., 1983). It is considered that this enzyme may be involved in the degradation of reserved starch where starch containing cells lack compartmental intactness (Buchner et al., 1996; Schupp and Ziegler, 2004).

Biochemical aspects

Kumar (1989) reviewed purification and immobilization of starch phosphorylase from various higher plant tissues. The enzyme has been purified from pea leaves, (Kruger and ap Rees, 1983; Conrads et al., 1986), *Zea mays* (Mateyka and Schnarrenberger, 1988), potato (Brisson et al., 1989), sweet potato (Chang et al., 1987), pea cotyledons (Myers and Matheson, 1991; Van Berkel et al.,1991), yam (Hamdan and Diopoh, 1991), red sea weeds (Yu and Pederson, 1991) rice seedling and red algae (Hsu et al., 2004: Yu et al., 1993).

The enzyme is generally inactivated rapidly above 55°C. The activity of potato phosphorylase has been shown to increase during low temperature storage (Kennedy and Isherwood, 1975). A number of regulatory mechanisms exist for the regulation of starch phosphorylase which include control by pH, temperature, redox potential, oligosaccharides level, allosteric changes and covalent modification (Smith et al., 2005; Orzechowski, 2008). Intracellular compartmentation is also thought to be indirect control on regulation (Brisson et al., 1989) as well as transcriptional level control is also suggested (St Pierre et al., 1996). Since all the metabolites of starch degradation are eventually exported to cytosol, it can be considered that starch degradation is regulated and feed back inhibition is exerted at the cellular level rather than at the individual level (Settke et al., 2007).

Stability of potato phosphorylase is reported at 30°C between pH 6 and 8 but is rapidly inactivated at pH 4.9 (Lee, 1960).

Optimum pH 6 has been reported for several multiple forms of starch phosphorylase from germinating pea seeds (Matheson and Richardson, 1976), tapioca tubers, tapioca leaves and banana leaves (Kumar and Sanwal, 1984, 1982a, b). Preiss et al. (1980), in case of spinach leaves, have reported optimum pH 6 to 7 for the cytoplasmic enzyme and pH 7.5 for the chloroplast starch phosphorylase. The optimum pH value of 6 to 7 has been reported for the enzyme (Conrads et al., 1986; Kamogawa

et al., 1968; Tsai and Nelson, 1969; Ariki and Fukui, 1975; Kumar, 1989). The optimum pH 6.5 has been reported for phosphorylase from corms of saffron crocus (Chrungoo and Farooq, 1993) and optimum pH 7 has been reported for both the multiple forms of maize starch phosphorylase during low temperature storage. Starch phosphorylases are generally inactivated rapidly above 55°C (Lee, 1960; Kamogawa et al., 1968).

Starch phosphorylase primarily acts as degrading enzyme for polyglucans via phosphorolysis and is mostly active in the homodimeric form with vitamin cofactor pyridoxal-5'-phosphate (Newgard et al., 1989; Hudson et al., 1993; Buchbinder et al., 2001). Although triose is observed to be the smallest primer required for its catalytic reaction for some of the starch phosphorylases, but in general, larger oligosaccharides have been found to be more efficient (Kumar 1989; Suganuma et al., 1991). There are reports of its primer-less activity, a characteristic that makes it one of the unique proteins. The primer less activity has been attributed to contamination of reagent with traces of oligosaccharides, the presence of glucosyl moieties in the enzyme, or due to some form of metabolic regulation (Venkaiah et al., 1991; Moreno and Tandecarz, 1996; Kossmann and Lloyd, 2000; Young et al., 2006).

Heavy metals such as Hg^{2+}, Ag^{+}, Cu^{2+}, Fe^{2+} and Zn^{2+} inhibit the activity of starch phosphorylase (Kumar, 1989; Hamdan and Diopoh, 1991). However, Mg^{2+} and Ca^{2+} act as effectors (Kastenschmidt et al., 1968). p-chloromereuribenzoate is inhibitory for potato tuber, banana leaves, tapioca leaves and tapioca tuber phosphorylases (Kumar, 1989).

Multiple forms of banana fruit phosphorylase are not inhibited by p-chloromercuribenzoate (Singh and Sanwal, 1976). However, ethylene diamine tetra acetic acid (EDTA) suppresses the activity of starch phosphorylase only at high concentration (Hamdan and Dipoh, 1991). Lee (1960) reported that potato phosphorylase does not contain serine phosphate and is unaffected by incubation with mammalian protein kinase, phosphate or AMP. In addition, neither ATP, DTT as reported by Gold et al. (1971) nor glucose-6-phosphate, fructose-6-phosphate, fructose-1, 6-bisphosphate, ribose-5-phosphate or fructose-1-phosphate activates the enzyme (Kamogawa et al., 1968). Lee and Braun (1973) also showed that sweet corn phosphorylase is unaffected by ATP or Mg^{2+}, but gets inhibited by ADP-glucose. Both the multiple forms of phosphorylase from *E. coli* are inhibited by sugar nucleotides, but not by AMP (Steup, 1988a). cAMP, AMP, glucose-1-phosphate, fructose-1, 6-bisphosphate, ADP-glucose, UDP-glucose and GDP-glucose have been shown to have no effect on *Klebsiella pneumoniae* malto-dextrin phosphorylase (Steup, 1988a).

However, granulose phosphorylase of *Clostridium pasteuranium* is inhibited by ADP-glucose, GDP-glucose, UDP-glucose and to a lesser extent by ATP, $NADP^{+}$, and NADPH, and AMP is found to be slightly stimulatory (Steup, 1988a). One form of loranthus leaf phosphorylase has been reported to be activated by ATP and AMP both (Khanna et al., 1971). On the other hand, banana leaf and fruit phosphorylases are not influenced by AMP but get inhibited by ATP (Kumar and Sanwal., 1981b, 1983a, b; Singh and Sanwal, 1976).

The P1 exhibited a strong binding affinity to immobilized glycogen upon electrophoresis whereas P2 and P3 did not exhibit strong binding affinity for immobilized glycogen. The cytosolic leaf phosphorylase has been purified to homogeneity by affinity chromatography. The single polypeptide product constituted both P1 and P2 activity forms. Probes for the detection of phosphorylase transcripts have been derived from cDNA sequences of cytosolic and plastidic phosphorylases, and these together with activity assays and a cytosolic phosphorylase specific antiserum have been used to monitor phosphorylase expression in leaves and seeds (Schupp and Zeigler, 2004).

The activity of rabbit glycogen phosphorylase has been reported to be regulated by a lipoprotein (Rutherford et al., 1986).

The phosphorylases from *Chlorella* have been reported to be activated by ammonium ions (Miyachi and Miyachi, 1987; Pan et al., 1988). It is also reported that β-amylase in sweet potato is an inhibitor of starch phosphorylase. Besides, aromatic amino acids viz. phenylalanine, tyrosine and tryptophan have been shown to be potent inhibitors of banana leaves (Kumar and Sanwal, 1983a), tapioca leaves (Kumar and Sanwal, 1982b), tapioca tubers (Kumar and Sanwal, 1984), one form of banana fruit phosphorylase (Singh and Sanwal, 1976). Since aromatic amino acids are originally derived from starch, it is believed that inhibition may exert a control via negative feedback mechanism ((Kumar and Sanwal, 1988).

Unlike animal phosphorylases, serine phosphate could not be detected in plant phosphorylases (Kumar, 1989). However, role of serine phosphate in animal phosphorylase is yet to be explained.

All the α-glucan phosphorylases either from animals, plants or microorganisms, contain pyridoxal phosphate as a prosthetic group (Graves and Wang, 1972; Iwata and Fukui, 1973; Steup, 1988; Kumar, 1989). On the other hand, Kumar and Sanwal (1982a, b) reported no pyridoxal phosphate in banana leaf and tapioca leaf starch phosphorylases. It has been explained that pyridoxal phosphate is involved neither in direct catalysis nor in regulation, but is present as a structural determinant essential for the enzyme activity (Kumar, 1989).

Starch phosphorylase and glycogen phosphorylase have a kinetic mechanism which involves random order of substrate with a rapid attainment of equilibrium and with inter-conversion of ternary complexes at rate limiting step (Steup and Schachtele, 1981; Kruger and ap Rees, 1983).

The Km value for the substrate depends not only on the temperature and pH but on the concentration of other

Table 1. General biochemical characteristics of the enzyme.

S. No.	Biochemical characteristics	Value
1	pH of the enzyme assay in the direction of starch (polysaccharide) synthesis	Around pH 6
2	pH of the enzyme assay in the direction of starch (polysaccharide) degradation	Around pH 7 to 7.5
3	pH stability	pH 6 to 8
4.	Temperature stability	Around 30°C, upper limit 55°C
4	Sub-unit molecular weight	Around 90,000 to 110,000 Da
5	Km for glucose -1 phosphate	0.6 to 5 mM
6	Km for inorganic phosphate	1 to 15 mM

substrate also. The values of Km for inorganic phosphate are normally in the range of 1 to 15 mM, and for glucose-1-phosphate normally in the range of 0.6 to 5 mM. The Km value of plastidic starch phosphorylase for glycogen and amylopectin in the direction of glucan synthesis are much higher than those of the cytoplasmic enzymes. Similar case is in the direction of phosphorolysis. However, Km values for the inorganic phosphate with both the enzymes are of the similar order. General biochemical characteristics of the enzyme are mentioned in Table 1.

Generally, all plant phosphorylases exhibit classical Michaelis kinetics but banana leaf phosphorylases showed biphasic kinetics indicating mixed positive-negative co-operativity and which is more prominent to the presence of aromatic amino acids. Aromatic amino acids are found to be inhibitors of banana leaf phosphorylases (Kumar and Sanwal., 1988). The multiple forms of phosphorylases are well differentiated by their kinetic behavior (Matheson and Rechardson, 1978; Steup et al., 1980a; Preiss et al., 1980; Steup and Schachtele, 1981; Shimomura et al., 1982; Kruger and ap Rees, 1983; Mateyka and Schnarrenberger, 1988; Myers and Matheson, 1991; Chrungoo and Farooq 1993).

Animal glycogen phosphorylases are regulated allosterically upon binding an effector or by covalent modulation at the N-terminal Ser14 residue by specific protein kinases. Two conformational states namely T (tight) and R (relaxed) are characterized by their inactive and active state, respectively (Johnson, 1992). Earlier, plant starch phosphorylases were being considered to be always active species only without any regulatory mechanism. Afterwards, this view of non-regulatory mechanism of starch phosphorylases, and in general for other starch-degrading enzymes has been found to be no longer true. A number of regulatory mechanisms for starch degrading enzymes have been shown like control by pH, redox potential, oligosaccharide level, allosteric control and covalent modulations (Smith et al., 2005; Orzechowski, 2008; Rathore et al., 2009). Studies indicated regulatory mechanisms such as intracellular compartmentation as an indirect control (Brisson et al., 1989) or at the transcriptional level (St Pierre et al., 1996) for starch phosphorylase. Fettke et al. (2007) argued that the starch degradation is regulated and feed back inhibition is exerted at the cellular level rather than at the individual organelles since all the metabolites of starch degradation are eventually exported to cytosol. Studies have shown that starch phosphorylase gets inhibited by aromatic amino acids (Kumar and Sanwal, 1983a, b, 1988; Venkaiah and Kumar, 1996). Inhibition of starch phosphorylase by aromatic amino acids has been correlated as feedback inhibition mechanism since basically aromatic amino acids are synthesized from starch (Kumar, 1988). Allosteric regulation of starch phosphorylases by L-tyrosine and ATP has also been reported (Singh and Sanwal, 1973; Kumar and Sanwal, 1983b, 1988).

Protein phosphorylation by kinase and dephosphorylation by phosphatase represents a ubiquitous mechanism of regulation for a variety of cellular processes. Tetlow et al. (2004a, b) demonstrated regulation by phosphorylation in starch branching enzyme and *Triticum aestivum* starch phosphorylase. There are reports that multiple forms of starch branching enzyme and Pho 1 are phosphorylated at one or more serine residues by plastidial protein kinase(s) in amyloplasts. Attempts are also done to search starch phosphorylase specific protein kinases. Young et al. (2006) reported a novel protein kinase that phosphorylates the L78 peptide of Pho 1 in sweet potato roots. However, this protein kinase does not phosphorylate Pho 2 and proteolytically modified Pho 1 lacking L78 indicating that this kinase is specific for L78 peptide of Pho 1 (Rathore et al., 2009). The molecular weight of this protein kinase has been reported to be nearly 338,000 (Young et al., 2006). This kinase phosphorylates only one seryl residue in L78 in spite of the fact that computational analyses showed many putative phosphorylation sites (Young et al., 2006; Rathore et al., 2009).

STRUCTURE AND FUNCTION

The animal counterpart of starch phosphorylase, glycogen phosphorylase is comparatively a more studied enzyme. Glycogen phosphorylase from rabbit muscle is especially most widely studied enzyme among all

glycogen phosphorylases. Over 100 structures of glycogen phosphorylases from several different sources, their mutants and complexes have been reported in Protein Data Bank (PDB, available at http://www.rcsb.org). It has been shown that temporarily blood glucose level in diabetic patients may be controlled by inhibiting glycogen phosphorylase enzyme activity. Although, it is a temporary solution but it has the advantage of no risk of hypoglycemia which is considered to be main side effect of several antidiabetic agents. Therefore, many studies are in progress searching for structure based drug discovery (Buchbinder et al., 2001; Oikonomakos, 2002, Rathore et al., 2009).

Amino acid composition of many animal phosphorylases and potato tuber phosphorylase has been reported (Kamogawa et al., 1968; Cohen et al., 1973). The amino acid composition of phosphorylases isolated from young and mature banana leaves is found to be quite same. However, it is not same in amino acid composition from rabbit muscle and potato phosphorylases (Kumar and Sanwal, 1982d).

Gene of *E. coli* α-glucan phosphorylase has been cloned and sequenced (Yu et al., 1988). Lin et al. (1991) has reported the primary structure of sweet potato starch phosphorylase deduced from its cDNA sequence. Mori et al. (1991) reported cloning and nucleotide sequencing of the gene coding for a potato tuber phosphorylase isozyme.

A few attempts have been made to clone the genes encoding plastidic and cytosolic phosphorylase (Kossmann and Frohberg, 2004; Schupp and Zeigner, 2004, Rathore et al., 2009). The plastidial phosphorylases are characterized by the presence of approximately 80 residue long insertion region in the centre known as L78 insertion region which was first identified in *Solanum tuberosum* (Nakano and Fukui, 1986). It is suggested that L78 segment might be derived from an intron ancestor (Chen et al., 2002). The oligomeric state of starch phosphorylase is homodimeric in its native state (Buchbinder et al., 2001), although heterodimeric or tetrameric forms have also been observed. In potato, two pho1 enzymes namely pho 1a (1.1) and pho 1b (1.2) exist (Sonnewald et al., 1995; Albrecht et al., 1998, 2001) and occur both in the homodimeric (pho1a) and heterodimeric (pho1a-pho1b) state (Albrecht et al., 1998). These enzymes are α-β proteins and belong to characteristic GT-B fold of glycosyl transferase enzyme (Breton et al., 2006). The monomeric unit contains two conserved N-terminal and C-terminal domains (Buchbinder et al., 2001). These two domains are thought to have arisen by the fusion of two ancestral genes each coding for a protein of approximately 50 KDa (Nakano and Fukui, 1986). Non vascular organisms such as unicellular green algae lack the insertion region in the pho1 (Dauvillee et al., 2006).

The L78 insert region is rich in ionic residues (Nakano and Fukui, 1986).

The sequences of amino acids and comparison of their primary structures have been reported (Palm et al., 1985; Nakano and Fukui, 1986; Yu et al., 1988, 1993; Mori et al., 1991). These studies indicated that α-1-4-glucan phosphorylase is an evolutionary well conserved protein. Kumar (1990) has predicted the secondary structure of maltodextrin phosphorylase from *E. coli* using the Chou Fasman model.

α - Glucan phosphorylases from plants, animals and prokaryotes occur as dimers and tetramers composed of apparently identical monomeric subunits (Fukui, 1982).

The apparent molecular weight of the monomers has been reported in the range of 100,000 Da (Graves and Wang, 1972). White potato tuber phosphorylase has been reported to have a native molecular weight 207,000 Da, as calculated from the sedimentation constant (Lee, 1960). There are two multiple forms in sweet potato having native molecular weight of 180,000 to 210,000 Da, respectively (Ariki and Fukui, 1975). The molecular weight of different multiple forms of potato phosphorylase as determined by gel filtration chromatography are 180,000, 320,000, 520,000 and 620,000 Da (Gerbrandy and Doorgeest, 1972). However, a molecular weight of 220,000 Da as calculated from the sedimentation coefficient has been reported for potato phosphorylase (Kamogawa et al., 1968; Iwata and Fukui, 1973).

Sweet corn phosphorylase has been reported to have the molecular weight of 315000 Da (Lee and Braun 1973). However, two phosphorylases purified from hybrid maize have been reported to have the molecular weight 160000 and 200000 Da, respectively (Burr and Nelson, 1975). Richardson and Metheson (1977) reported two multiple forms of phosphorylase along with their molecular weights in alaska pea cotyledons, banana fruit, broad bean, mung bean, tobacco leaf, pea cotyledons and potato tuber. One of the multiple forms has been reported to have the molecular weight in the range of 170,000 to 200,000 Da, whereas the molecular weight of other form is reported in the range of 330,000 to 490,000 Da.

Spinach leaf cytoplasmic and chloroplastic phosphorylases have been reported to have the molecular weight, 194,000 and 203,000 Da, respectively (Preiss et al., 1980). Kumar and Sanwal (1977) reported the molecular weights of 450000 and 220000 Da, respectively for two multiple forms present in banana leaves.

Hamdon and Diopah (1991) reported the molecular weight of yam starch phosphorylase to be 200000 Da. In *Gracilaria sordida*, molecular weight has been reported to be 243000 and 337000 Da, respectively (Yu and Pedersen, 1991a). Insect flight muscle phosphorylase multiple forms have molecular weights 320,000, 140,000 and 100000, respectively (Childress and Secktor, 1970). The molecular weight of pea leaf cytoplasmic phosphorylase is 160000 Da, the subunit molecular weight being 88,700 Da (Conrads et al., 1986). The cytoplasmic starch phosphorylase of maize has molecular weight of 195,000 Da for the native enzyme and 87,000 and

57,000 Da for the SDS treated enzyme protein.

This implies a dimeric structure for cytoplasmic starch phosphorylase and a tetrameric structure for the chloroplastic starch phosphorylase (Mateyka and Schnarrenberger 1988), Kumar and Sanwal (1981a, b) have reported subunit molecular weight of 55000 ± 3000 in case of mature banana leaf phosphorylase. It was shown that the enzyme is made up of identical polypeptide chains of molecular weight 110000 and on heating, there is splitting of the chain from the middle, similarly as has been assumed in the case of sweet potato phosphorylase (Ariki and Fukui, 1975). Pho 1 is susceptible to degradation either by proteolysis or by heating. The monomeric unit of mature banana (*Musa paradisiaca*) and tapioca (*Manihot utilissima)* leaf phosphorylases and L-form of starch phosphorylase from potato and sweet potato upon proteolysis dissociate into two subunits of 55,000 size, corresponding to two N- and C-terminal domains (Kumar and Sanwal, 1981b, 1982b; Brisson et al., 1989; Chen et al., 2002). It has been reported that starch phosphorylase retains its quaternary structure as well as full catalytic activity indicating the possible mechanism of proteolytically driven regulation of its activity (Chen et al., 2002; Young et al., 2006). The characteristic degradation susceptibility of Pho 1 as against Pho 2 suggested that L-78 peptide is the primary target of proteolytic degradation. The sequence analysis of this L78 peptide showed the occurrence of PEST region (rich in proline, glutamic acid, serine and threonine). The PEST regions are shown to occur in proteins that are degraded rapidly (Rogers et al., 1986). These are generally flanked by many positively charged amino acids clusters (Chen et al., 2002; Rathore et al., 2009).

As mentioned earlier, comparatively plant starch phosphorylases have been lesser studied. To the best of our knowledge, no crystal structure of either L- or H- form has been reported yet. Many people including our laboratory are engaged in studies related to determi-nation of crystal structures of starch phosphorylases aiming to elucidate the structure and intriguing role of L78 region. Computational modeling studies are also in progress. The computational modeling work has the advantage of probing the dynamics of this region. However, it has been mentioned that the scope of such studies is limited due to many factors. The L78 loop requires *ab inito* modeling - a challenging task given the size of the fragment. The details of computationally modeled structure vary depending on the choice of the template – unregulated or regulated structures and the latter in active or inactive states (Rathore et al., 2009). Besides, orientation of momoners in the dimeric structure, and the details of dimeric interface also vary among phosphorylases (Watson et al., 1997).

There is a report of crystal structure in PDB carrying the name of starch phosphorylase (referred as StP; PDB code: '24 cm') from soil bacterium *Corynebacterium callunae*. It is a crystallographic tetramer. This starch phosphorylase has been reported to belong to non-regulatory class of α-glucan phosphorylase and has preference for starch over glycogen and has also been mentioned as the first bacterial starch phosphorylase (Weinhäusel et al., 1997). This bacterial starch phosphorylase like other prokaryotic phosphorylases does not have L78 region in its structure. This bacterial starch phosphorylase may be considered to have important biotechnological application since its stability may be increased using conformational engineering approach (Nidetzky et al., 2003). It has been shown that upon phosphate ion binding with the enzyme protein, there is much stabilization and therefore this property can be exploited for studies on manipulation via conformational engineering. Griessler et al. (2004) probed the forces which stabilizes its quaternary structure.

It has been shown that all the α-glucan phosphorylases have conserved catalytic and structural features, and pyridoxal phosphate is an essential cofactor for catalytic activity. It is also shown that 5'-phosphate moiety in pyridoxal phosphate is the main catalytic domain (Palm et al., 1990; Buchbinder et al., 2001). However, Kumar and Sanwal (1982a, b) in case of banana and tapioca leaves starch phosphorylases reported absence of pyridoxal phosphate and still are catalytically active. It has been shown that pyridoxal phosphate is attached to conserved lysyl residue side chain in the active site through schiff base in between its 4'-aldehyde group and ε- amino group of lysine. It is also shown that functional oligomeric state of starch phosphorylase is homodimeric state (Buchbinder et al., 2001). Of course, tetrameric and heterodimeric states have also been observed. In potato, two Pho1 enzymic forms [Pho1a (L1) and Pho1b (L2)] have been observed (Rathore et al., 2009; Sonnewald et al., 1995; Albrecht et al., 2001). It is also shown that Pho1 occurs both in homodimeric and heterodimeric form (Albrecht et al., 1998). Breton et al. (2006) reported that these proteins are α/β proteins and belong to the GT-B fold of glycosyl transferase enzymes. The monomeric subunit has two conserved N-terminal and C-terminal domains (Buchbinder et al., 2001). It is also shown that the N-terminal domain contains most of the ligand binding residues and therefore is called as regulatory domain, and C-terminal domain contains most of the active site residues and pyridoxal phosphate binding residues, and therefore is called as catalytic domain. It is also predicted that the active site is located in between the N-terminal and C-terminal domains and regulatory domain has residues involved in dimeric contacts and are responsible for transmitting conformational changes across subunits upon ligand binding in regulated phosphorylases. These two domains are thought to have possibly evolved by the fusion of two ancestral genes each coding for a protein of nearly 50,000 molecular weight (Nakano and Fukui, 1986). Hudson et al. (1993) reported that the residues in the functional and dimeric interface sites in starch

phosphorylase and all other glucan phosphorylases are highly conserved. In non-vascular organisms viz. unicellular green algae, *Chlamydomonas reinhardtii*, Dauvillee et al. (2006) reported absence of insertion region of L78.

It has also been shown that Pho1 like other plastidial starch metabolizing enzymes also possess N-terminal transit peptide, in the precursor protein which gets cleaved upon translocation. The L78 insert region is rich in ionic residues (Nakano and Fukui, 1986). The transit peptide, L78 and its flanking proline-rich C-peptides are highly diverse. Excluding these regions, Pho1 enzymes share high sequence identity (~80%). Molecular modeling has suggested that the adjacent L78 loop might sterically block the active site resulting in low affinity for the substrates (Nakano and Fukui, 1986). The functional role of L78 insert has been studied by constructing chimeric enzymes (Mori et al., 1993a, b). Removal of L78 from L-type makes it completely inactive. However, the replacement of L78 region and flanking residues by the corresponding residues of Pho2 or the rabbit muscle phosphorylase containing glycogen storage site, showed its significantly high affinity for branched glucans.

Possible role of plastidic phosphorylases

The exact *in vivo* roles of plastidial phosphorylases are not yet confirmed. On the basis of indirect evidences, it has been said that plastidial phosphorylases are involved in phosphorolytic starch degradation. However, still many questions are unanswered. Sonnewald et al. (1995) demonstrated that the antisense repression of starch phosphorylase activity indicated no significant influence on starch accumulation in the leaves of transgenic potato plants. The T-DNA insertion in Pho1 gene of *Arabidopsis* suggested for an alternative route of starch phosphorylase (Zeeman et al., 2004b). The loss of plastidial starch phosphorylase does not cause a significant change in the level of starch accumulation in the day time or its mobilization at night. These results indicated that the absence of plastidial starch phosphorylase has no effect on starch degradation. However, it has been demonstrated that starch phosphorylase plays a role in stress tolerance pathway. The exposure to transient dehydration stress leads to the activation of starch phosphorylase that in turn triggers starch degradation. It has been argued that during stress, phosphorolytic starch degradation serves as a swift response to alleviate stress by feeding substrates into oxidative pentose phosphate pathway (Zeeman et al., 2004b; Lloyd et al., 2005).

On the other hand, there are evidences that plastidial phosphorylase (Pho 1) contributes in starch synthesis as expression and activity of plastidial starch phosphorylase correlates with starch synthesis (Brisson et al., 1989; Sonnewald et al., 1995; Yu et al., 2001; Tetlow et al., 2004b; Hannah and James, 2008). Albrecht et al. (2001) showed involvement of Pho 1a in starch synthesis in *Solanum tuberosum*. Dauvillee et al. (2006) showed involvement of plastidial phosphorylase Pho A and Pho B in starch synthesis.

d'Hulst et al. (2007) demonstrated that the switching off a gene of starch phosphorylase in many transgenic plants increases starch grain size and the amount of starch. Satoh et al. (2008) showed that Pho1 plays a crucial role in starch biosynthesis in rice endosperm, and it appreciably affects the starch structure. It remains unsolved, what mechanism exactly underlies the starch phosphorylase driven starch biosynthesis (Rathore et al., 2009). Other groups showed that disproportionating enzymes (D-enzymes; EC 2.4.1.25), in association with starch phosphorylase may contribute to overall starch synthesis by recycling the intermediate glucans, released in the process (Takaha et al., 1998a; Tetlow et al., 2004b). Colleoni et al. (1999) demonstrated that D-enzymes stimulate the phosphorolytic reaction of phosphorylase. According to glucan trimming model, it has been speculated that during discontinuous synthesis of starch granules, the short glucan chains released from pre-amylopectin by the action of debranching enzymes are converted to longer glucan chains by D-enzymes (Ball et al., 1996; Ball and Morell, 2003). The long glucan chains are then substrates for starch phosphorylase. The released glucose-1-phosphate is used up by ADP-glucose pyrophosphorylase (EC 2.7.7.27) for starch synthesis. Therefore, it may be said that reaction of starch phosphorylase in the direction of starch degradation ultimately contributes to its overall synthesis of starch. Reconciliation of the multiple lines of evidences on specific role of starch phosphorylase in starch metabolism requires new thinking and experiments. Experiments on the predicted role of proteolytically driven L78, acting as switch for synthesis or degradation are important (Chen et al., 2002). Others have proposed multi-protein complex machinery for starch metabolism. Studies have shown that the phosphorylation of starch phosphorylase and branching enzymes promote selective protein-protein interactions leading to the multi-protein complex, and this assembly is essential for starch synthesis and degradation (Tetlow et al., 2004a, b; Hennen-Bierwagen et al., 2009).

Possible roles of cytosolic phosphorylases

The physiological substrate and the biological function(s) of cytosolic phosphorylase have remained unsolved for a long time. The controversy surrounded over the exact role of cytoplasmic starch phosphorylase since starch granules are found inside the chloroplasts or amyloplasts and are impermeable to the surrounding membranes. The elimination studies in *Solenum tuberosum* indicated that it had no effect on starch degradation. However, studies on anti-sense inhibition of cytosolic

phosphorylase indicated that it has effect on sprout growth and flowering (Duwenig et al., 1997). Steup et al. (1983) showed that the cytosolic Pho2 lacks Pho1 type of L78 insert, which sterically hinders the substrate binding and therefore they are more effective in degrading large branched glucans, and may even attack starch granules. Therefore, it has also been predicted that this enzyme is involved in starch degradation of reserve starch in plant organs where the starch containing cells have lost their compartmental integrity, such as in the cotyledons of germinating legumes (Buchner et al., 1996; Schupp and Ziegler, 2004). On the other hand, in cells with intact plastids, cytosolic phosphorylase may be involved in metabolism of products of starch degradation, exported from the chloroplast and the regulation of cytosolic glucose-1-phosphate level.

Steup group demonstrated that the cytosolic phosphorylase functions in a novel glycan metabolism. The experiments on pea (*Pisum sativam*) and *Arabidopsis* indicated that soluble heteroglycans (SHG) with a complex pattern of glycosidic linkages are the substrates for the cytosolic phosphorylase (Fettke et al., 2004, 2005b; Lu et al., 2006). Fettke et al. (2005a) showed that in transgenic potato plants, anti-sense inhibition of Pho2 resulted in increased glucosyl and rhamnosyl contents of the glycans, whereas its over-expression decreased their contents. This is perhaps the first *in vivo* indication for the physiological substrate of the cytosolic phosphorylase. Lu et al. (2006) demonstrated that cytosolic AtPHS2 (Pho2) and disproportionating enzymes (DPE2) in *Arabidopsis* act on maltose and SHGs, akin to amylomaltase and maltodextrin phosphorylase orthologs in *E. coli*. The T-DNA insertion line of the AtPHS2 gene increased the night time maltose level. Chia et al. (2004) showed that in DPE2 deficient mutants of *Arabidopsis*, the activity of the cytosolic phosphorylase increased, indicating the role of the enzyme as a buffer between starch degradation and cytosolic metabolism.

Biotechnological aspects/ industrial applications

The α- glucan phosphorylase can be used for the production of glucose-1-phosphate, to synthesize amylose and a variety of novel glucans for designing starch and development of transgenic varieties with modified starches (Fujii et al., 2003, 2007; Kossmann and Frohberg, 2004; Morell and Myers, 2005; Yanase et al., 2006a, 2007; d'Hulst et al., 2007; van der Vlist et al., 2008; Rathore et al., 2009). Glucose-1-phosphate is an important metabolite and has been used in cardiotherapy as a cytostatic compound (Venkaiah and Kumar, 1994). Glucose-1-phosphate has also been used as an antibacterial, anti-inflammatory and anti-tumor agent (Shin et al., 2000; Sakata et al., 2007; Rathore et al., 2009). It has also been used for the chemical synthesis of glucuronic acid and trehalose production (Takahashi et al., 1996; Rathore et al., 2009).

Amylose is a functional biomaterial and is also used to produce cycloamylose/ cyclodextrin and other glucose polymers. Amylose is not available as an industrial raw material, since its separation from amylopectin in starch is difficult and has a low yield. As the molecular size of amylose can be controlled by glucose-1-phosphate / primer molar ratio, the starch phosphorylase may be a potentially good system for its manufacturing. Since starch phosphorylase can synthesize only amylose, desired characteristic ratio of amylose to amylopectin in industrial starch can be manipulated. Amylose can also be used as inclusion compounds and has applications in drug delivery and nanotechnology (Ohdan et al., 2006, 2007a). The synthetic amylose can be designed with more mechanical strength and better moldability by controlling molecular size and branching and therefore it can be used as biodegradable plastics (Masao et al., 2005). Starch phosphorylase has been used for increased storage life of potato as the reduction of phosphorylase content in potato tubers leads to a substantial decrease in the accumulation of sugars (Kawchuk et al., 1999). On inactivation of starch phosphorylase, it has been observed that there is increase in the starch grain size in transgenic plant. Therefore, it is useful for increasing yields in the extraction and purification of starch on an industrial scale since smaller starch grains are generally lost during washing (d'Hulst et al., 2007). Starch phosphorylase is also used as a biosensor to detect amount of inorganic phosphate pollution in the environment and in the micro-estimation of inorganic phosphate under pathological conditions (Hüwell et al., 1997).

The glucan production in an cheaper way has been made possible by the joint use of α-glucan phosphorylase and other glucan enzymes. Its biochemical production using glucose-1-phosphate at the industrial scale is costly (Rathore et al., 2009). This has been accomplished in two different manners viz. by phosphorolysis of sucrose or cellobiose by sucrose phosphorylase (EC 2.4.1.7) or cellobiose phosphorylase (EC 2.4.1.20) respectively in the presence of inorganic phosphate. The joint system of sucrose phosphorylase, glucan phosphorylase and cellobiose phosphorylase as well as glucan phosphorylase has been used for enzymatic industrial production of amylose (Ohdan et al., 2006, 2007a, b; Yanase et al., 2006a; Fujii et al., 2007; Rathore et al., 2009).

The immobilized starch phosphorylase can be used for the synthesis of glucose-1-phosphate. It can also be used in microestimation of inorganic phosphate under pathological conditions and also to monitor the environmental pollution. Starch phosphorylase for the first time was immobilized on polyacrylamide by Kumar and Sanwal in 1981. In the past few years, immobilized starch phosphorylase or starch phosphorylase co-immobilized with other enzymes has been exploited for many

purposes. Sucrose production from starch and fructose has been reported using co-immobilized starch phosphorylase, amylosucrase and sucrose phosphorylase enzyme bed reactor (Daurat et al., 1982). Szulczynski (1986) reported immobilization of starch phosphorylase on chitin derivatives.

Venkaiah and Kumar (1994, 1995) immobilized starch phosphorylase from sorghum leaves and potato waste water and developed bioreactors for the production of glucose-1-phosphate. They used various solid supports viz. egg shell, gelatin, alumina. Srivastava et al. (1996) immobilized starch phosphorylase from *Cuscuta reflexa* filaments using egg shell as solid support. Upadhye and Kumar (1996) immobilized starch phosphorylase from bengal gram seeds using alginate beads and DEAE cellulose as solid support.

The immobilization of starch phosphorylase from Indian millet has been reported for production of glucose-1-phosphate (Garg and Kumar, 2007). They used brick dust as solid support Garg and Kumar (2008) also reported immobilization of starch phosphorylase from cabbage leaves on egg shell and showed these immobilized enzymes as very good for production of glucose-1-phosphate.

CONCLUSION

Starch phosphorylase plays a dynamic role between starch synthesis and degradation. However, plant starch phosphorylase is largely considered to be involved in phosphorolytic degradation of starch. It reversibly catalyzes the transfer of glucosyl units from glucose-1-phosphate to the non-reducing end of glucan chain with the release of inorganic phosphate. It is widely distributed in plant kingdom. Enzyme multiplicity is also common in starch phosphorylase and different multiple forms have been predicted to have different roles in starch metabolism. Various biochemical properties have been reviewed. Its regulation by aromatic amino acids has also been discussed. Importance of plastidial and cytoplasmic starch phosphorylase has also been discussed. Various biotechnological aspects and industrial applications have been reviewed. Its exploitation in production of glucose-1-phosphate, a cytostatic compound has been discussed in the present review. Amylose is a functional biomaterial and is also used to produce cycloamylose/cyclodextrin and other glucose polymers. Amylose is not available as an industrial raw material, since its separation from amylopectin in starch is difficult and has a low yield. As the molecular size of amylose can be controlled by glucose-1-phosphate/primer molar ratio, starch phosphorylase may be a potentially good system for its manufacturing. Since starch phosphorylase can synthesize only amylose, desired characteristic ratio of amylose to amylopectin in industrial starch can be manipulated. Amylose can also be used as inclusion compounds and has applications in drug delivery and nanotechnology. The synthetic amylose can be designed with more mechanical strength and better moldability by controlling molecular size and branching, and therefore it can be used as biodegradable plastics. Starch phosphorylase is also used as a biosensor to detect amount of inorganic phosphate pollution in the environment and in the micro-estimation of inorganic phosphate under pathological conditions.

ACKNOWLEDGEMENTS

The authors acknowledge the facilities of the Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi (DBT) under the Bioinformatics sub centre used in the preparation of the manuscript. The authors also acknowledge UGC, New Delhi for partial financial support.

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