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Purification and Characterization of α-Glucan Phosphorylase Isoform Pho 2 from Spinach Leaves

Ritu Jain[1] and Anil Kumar[1*]

[1]School of Biotechnology, Devi Ahilya University, Khandwa Road, Indore-452001, India.

Author's contributions

This work was carried out in collaboration between all authors. Author RJ carried the experimental work and wrote the first draft of the manuscript and. Author AK designed the study, managed literature searches, corrected the entire manuscript, performed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

α-Glucan phosphorylase is an important enzyme of carbohydrate metabolism. In spinach leaves, it has been reported in two multiple forms viz. Pho 2 (cytosolic) and Pho 1 (plastidial). Here, we extracted and purified Pho 2 form of α-glucan phosphorylase from spinach using salting out with ammonium sulfate, desalting using Sephadex G-25 chromatography, anion exchange chromatography using DEAE-Sepharose and gel filtration chromatography using Sepharose-4-B. The purified enzyme had a specific activity, 150 units/mg protein. There was 38% recovery and 652 fold purification after final Sepharose-4B chromatography. The purified enzyme showed a single protein band on SDS sodium dodecyl sulfate polyacrylamide gel electrophoresis having molecular weight 94,000±2000 daltons. The native molecular weight is found to be 188,000±3000 daltons as determined using gel filtration chromatography over Sephadex G-200. The Pho2 exhibited optimum pH at pH 6.0 with two half pH optima at pH 5.2 and pH 7.0. The optimum temperature of Pho2 is found to be 37ºC with two half temperature optima at 30ºC and 40ºC. The Km value of the enzyme for starch and glucose-1-phosphate is found to be 116 µg/mL and 0.55 mM, respectively.

Keywords: α-Glucan phosphorylase; Pho 2; spinach leaves; gel filtration chromatography; Km values.

**Corresponding author: Email: ak_sbt@yahoo.com;*

1. INTRODUCTION

α-Glucan phosphorylase (α-1,4-D-glucan: orthophosphate, α-D-glucosyl transferase; E.C.2.4.1.1), also called starch phosphorylase, belongs to the family of glycosyl transferase, one of the largest families of carbohydrate metabolism. Multiple forms of starch phosphorylase have been shown in various plant tissues such as pea leaves, bundle sheath cells of maize leaf, banana fruit pulp, germinating pea seeds, potato tubers, tobacco leaf, *Arabidopsis* leaf, spinach leaves [1-8]. As in other plants, in spinach (*Spinacea oleracea*) leaves too, two multiple forms of starch phosphorylase have been reported and designated as Pho 1(L-form) and Pho 2 (H-form) [9]. The intracellular localization of Pho1 is in chloroplast whereas Pho 2 is in the cytoplasm.

The precise biological function of the cytoplasmic phosphorylase is still not well understood. Since starch granules are found inside the chloroplasts or amyloplasts and both starch and Pho2 are impermeable to the surrounding membranes, Pho2 does not have any direct access to starch. It has been shown using genetic inhibition that the cytoplasmic form has no major role in starch degradation and rather Pho2 somehow regulates sprout growth and flowering in some plants such as *Arabidopsis* [10].

The cytoplasmic form Pho2 lacks the L-78 insert, characteristic of the plastidial form. The absence of this region has been reasoned as the cause for the high affinity of Pho2 for both linear and branched glucans, and because of the same reason Pho2 is considered to be the analogue of glycogen phosphorylase found in animals and microbes. Due to its high affinity for glucans, it has been argued that this enzyme may be involved in starch degradation of reserve starch in plant organs where the starch containing cells have lost their intracellular compartmentalization, such as in the cotyledons of germinating legumes [11]. While, in cells with intact plastids, such as in leaves, cytoplasmic phosphorylase may be involved in the metabolism of products of starch degradation, exported from the chloroplast and the regulation of cytoplasmic glucose-1-phosphate level [12]. It has been shown that soluble heteroglycans with a complex pattern of glycosidic linkages are the substrates for the cytoplasmic phosphorylase [13]. In fact, it has also been shown through insertional mutagenesis in *Arabidopsis* that Pho2 (AtPHS2 of *Arabidopsis*) along with DPE2 (cytosolic transglucosidase; EC 2.4.1.25) involves in maltose metabolism. DPE2 generates heteroglycans from maltose and heteroglycans serve as substrate for Pho2 and thus Pho2 may be regarded as the plant analogue of *E. coli* maltodextrin phosphorylase (MalP) [14]. Ma et al. [15] reported multiple alignment of the genes of starch phosphorylase and showed that both the genes of all species have 15 exons and 14 introns and the ligand binding sites at exon-intron junction is highly conserved as compared to other regions. They also reported that both Pho1 and Pho2 are homologous except two regions viz. a N-terminal domain and another is L-78 region. Considering the importance of α-Glucan phosphorylase in starch metabolism, here we have studied purification and characterization of Pho 2 from spinach leaves. Authors are in the process of doing further structural studies on starch phosphorylase. Spinach leaf starch phosphorylase was selected since it has been biochemically characterized earlier also.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh spinach leaves were purchased from the local market. The spinach leaves were washed thoroughly under running tap water and subsequently with distilled water to remove any adhering soil or other impurity. Thereafter, adhered water was soaked gently pressing the spinach leaves between two filter papers.

2.2 Enzyme Homogenate

Washed spinach leaves (10 g) were deveined and frozen in liquid nitrogen. Thereafter, frozen leaves were crushed to fine powder using pestle and mortar. The powder was gently suspended using a glass rod in 90 mL of chilled extraction buffer containing 50 mM Imidazole-HCl buffer, pH 7.5, 1 mM dithiothreitol, 1 mM Na_2-EDTA, 0.1 mM phenyl methyl sulfonyl fluoride (PMSF), and 10% glycerol. The suspension was filtered through four layers of muslin cloth and the filtrate was subjected to centrifugation for 20 minutes at 15000 x *g* at 0 to 4ºC.

2.3 Purification of the Cytosolic α-Glucan Phosphorylase

The cytosolic α-Glucan phosphorylase was purified using following steps at 0 to 4ºC in a cold room.

2.3.1 Salting out using ammonium sulfate

To the enzyme homogenate, powdered ammonium sulfate was added with constant stirring to get 40% saturation. The pH was maintained at 7.5 by adding dilute ammonia (1% v/v). The resulting suspension was incubated for 4 hours at 0-4ºC. Thereafter, the pellet was collected after centrifugation at 15000 x *g* for 15 minutes and discarded. Powdered ammonium sulfate was added to the supernatant, which had most of the enzyme activity, to 40 to 65% saturation and incubated overnight at 0-4ºC. Thereafter, pellet was collected after centrifugation at 15000 x *g* for 15 minutes. The pellet obtained was dissolved in minimal volume (10 mL) of buffer-A (20 mM citrate-NaOH, pH 6.5, 0.5 mM DTT, and 10% glycerol).

2.3.2 Desalting using sephadex G-25 gel filtration chromatography

The dissolved pellet in buffer A was centrifuged at 15000 x *g* for 10 minutes to get clear supernatant which was subjected to Sephadex G-25 chromatography using a column (50 cm X 1.8 cm) pre-equilibrated with buffer-A. The flow rate of the column was adjusted nearly 2 mL/ minute. After discarding the void volume, 5 mL volume fractions were collected and analyzed for enzyme activity. The fractions having enzyme activity were pooled.

2.3.3 Anion-exchange chromatography

The pooled fraction after Sephadex G-25 chromatography was applied on to a DEAE-Sepharose column (15 cm X 0.9 cm) pre-equilibrated with buffer-A. After applying the sample, the column was washed with 100 mL of the same buffer. Thereafter, the enzyme was eluted using a linear gradient consisting of 100 mL of buffer-A in the mixing chamber and 100 mL of buffer-B (60 mM Citrate-NaOH, pH 6.5, 0.5 mM DTT, and 10% glycerol) in the reservoir. The concentration of citrate buffer was calculated from the following formula [16]:

$$C_v = C_2 + (C_1 - C_2).\ v/V_{total}$$

Where,

C_v = concentration of citrate buffer present in the effluent after v volume has flowed through it.
C_1=concentration of citrate buffer initially present in the reservoir.
C_2=concentration of citrate buffer initially present in the mixing chamber.
V_{total}= total volume of liquid present in both the containers

The fractions of phosphorylase activity peak were pooled and the pooled fraction was used for further purification by gel filtration chromatography. Before loading on Sepharose-4B column, to the pooled fraction, solid ammonium sulfate was added with constant but gentle shaking to get 90% saturation. After storage for 4 hours in the cold condition, the suspension was centrifuged at 15,000 x *g* for 20 minutes to collect the precipitate which was dissolved in minimal volume of buffer C (HEPES-NaOH buffer, pH 7.0 containing 0.5 mM DTT, 1 mM EDTA, and 10% glycerol). The suspended fraction was centrifuged at 15,000 x *g* for 15 minutes to get clear supernatant.

2.3.4 Sepharose-4B gel filtration chromatography

The clear supernatant after concentrating using ammonium sulfate, was loaded onto a Sepharose-4B column (30 cm x 1.6 cm) pre-equilibrated with buffer C. After discarding the void volume, fractions of 2 mL were collected and analyzed for the enzyme activity. The active fractions were pooled as a single fraction and used for characterization.

2.4 Enzyme Assay

The enzyme assay was carried out in the direction of polysaccharide synthesis as described by Garg and Kumar [17] with some modifications. The enzyme assay system was consisted of 200 µl of 0.2 M tris-maleate buffer, pH 6.0; 100 µl of 0.02 M sodium fluoride; 100 µl of 3% soluble starch and 500 µl of the enzyme preparation and water, pre-incubated at 37ºC for 2 min. The reaction was started by the addition of 100 µl of 50 mM glucose-1-phosphate. After 30 min, the reaction was stopped by the addition of 100 µl of 50% TCA and the tubes were put in an ice bath for 2 minutes. The precipitate formed was removed by centrifugation at 0 to 4ºC. In the clear supernatant, inorganic phosphate formed was estimated using colorimetric method of Fiske and Subbarow [18]. One unit of the enzyme activity was taken as the amount of the enzyme required to liberate one micromole of inorganic phosphate in one minute under the conditions of the enzyme assay. Specific activity was defined as units per mg protein.

2.5 Protein Estimation

Protein estimation was done according to the method of Lowry et al. [19] as modified by Khanna et al. [20] using bovine serum albumin as the standard.

2.6 Zymogram Analysis

Non-denaturing polyacrylamide gel electrophoresis was done according to the procedure of Gabriel [21] using 5% acrylamide in slab gels (1 mm thick) except for the modification that glycogen (0.2 % w/v) was incorporated in the separating gel as a primer for the enzyme. About 70-80 µg of total protein was loaded in each sample well. The buffer systems used were 0.2 M Tris-HCl, pH 8.8 for separating gel and 0.2 M Tris-HCl pH 6.8 for the stacking gel. Electrophoresis was carried out using a current of 3 mA/cm. The current was turned off when the tracking dye (bromophenol blue) reached at the bottom of the gel. Time of reaching the tracking dye at the bottom of the gel was determined in a separate experiment. After electrophoresis, gel was incubated overnight in an incubation mixture containing 20 mM glucose-1-phosphate in 20 mM Tris- maleate buffer, pH 6.0 at 37ºC with constant shaking. The enzyme activity band(s) was detected by the glucan synthesized at the site of the enzyme band and presence of glucan deposited was detected by staining with Lugol solution containing 0.1% w/v Iodine, 1% Potassium Iodide and 3% v/v HCl. On staining with Lugol solution, glucan deposited was indicated by reddish band(s).

2.7 Native Molecular Weight Determination

Native molecular weight of Pho 2 was determined using Sephadex G-200 gel filtration chromatography. For that, purified Pho 2 enzyme protein was loaded onto a Sephadex G-200 column (1.6 x 50 cm) pre-equilibrated with buffer C. After loading, chromatography was carried out using buffer C and fractions of 3 mL were collected at a flow rate of nearly 10 mL/ hour. Standard molecular weight markers namely thyroglobulin (MW 669,000), apoferritin (MW 443,000), β-amylase (MW 200,000) and albumin (MW 66,000) were also chromatographed using the same column. A standard graph was plotted using semi-logarithmic graph between elution volumes versus log molecular weight of standard proteins. From the elution volume of Pho 2, molecular weight was calculated from the graph [22].

2.8 Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis was carried out according to the procedure of Laemmli [23] with some modifications. In the resolving gel, the buffer system was consisted of 0.2 M Tris-HCl, pH 8.8 and in the stacking gel was 0.2 M Tris-HCl buffer, pH 6.8. The gels were 8 % acrylamide and 1 mm thick. The electrode buffer used was 0.05 M Tris-HCl, pH 8.8. The protein samples were mixed with equal volume of sample buffer containing 62.5 mM Tris-HCl buffer, pH 6.8, 2% w/v SDS, 20 mM DTT, 10% glycerol and 0.003% w/v bromophenol blue and were heated in a boiling water bath for 5 minutes. About 10-15 µg protein in each sample, were loaded in a sample well. Electrophoresis was carried out using a current of 3mA/ cm. The current was turned off when the tracking dye (bromophenol blue) reached near the bottom of the gel. The gels were stained using the staining procedure of Fairbanks et al. [24]. To detect very low amounts of protein, silver staining was also used occasionally. For molecular weight determination, standard molecular markers mid range with molecular weight range of 34 to 200 kDa were used. Coomassie brilliant blue (0.2% in methanol, acetic acid and water in the raio of 3:1:6) was used for staining the protein bands.

2.9 Characterization

2.9.1 Optimum pH

The purified Pho 2 enzyme was assayed using 0.2 M Tris-maleate buffer of different pH ranging from pH 4.5 to pH 8.0 to determine the optimum pH at which the Pho 2 exhibits maximum activity.

2.9.2 Optimum temperature

Optimum temperature of the Pho2 enzyme was determined by assaying the enzyme at different temperatures ranging from 10 and 60ºC.

2.9.3 Determination of the effect of aromatic amino acids on enzyme activity

The enzyme assays were carried out in the presence of 1 to 6 mM tryptophan or phenylalanine, in 0.2 M Tris-maleate pH 6.0. In the control tubes, enzyme assay was carried out in the absence of any aromatic amino acid as described above.

2.9.4 Determination of K_m and V_{max} for starch and glucose-1-phosphate

For determination of K_m and V_{max}, the enzyme assays were carried out using different concentrations of one substrate keeping the other constant. For determination of Km for starch, different concentrations of starch used were 0.06, 0.08, 0.10, 0.15, 0.20 and 0.40 mg/mL, whereas to determine Km for glucose-1-phosphate, concentrations of glucose-1-phosphate used were 0.12, 0.25, 0.40, 0.50, 0.80 and 1.0 mM glucose-1-phosphate.

3. RESULTS AND DISCUSSION

3.1 Purification of Pho 2

3.1.1 Enzyme homogenate

In 100 mL of 10% homogenate preparation of spinach leaves, the total protein content was found to be 34 mg and the total enzyme activity was 7.8 units. The specific activity of starch phosphorylase in the enzyme homogenate was found to be 0.23 unit/mg protein.

3.1.2 Ammonium sulfate fractionation and desalting

Most of the enzyme activity was found in the 40-65% ammonium sulfate fraction. The total protein content of the fraction was 18 mg protein and the total enzyme activity was 7.6 units and therefore the specific activity was 0.42 unit/mg protein. The total recovery of both the forms of the enzyme was 97%. After desalting through Sephadex G-25 chromatography, more than 90% of the salting out fraction was recovered as shown in the purification profile (Table 1). Zymogram analysis showed two bands of enzyme activity as shown in Fig. 1 and thus the enzyme activity calculated corresponded to both the multiple forms.

3.1.3 DEAE-Sepharose ion-exchange chromatography

The elution profile of Pho 2 on ion-exchange chromatography using DEAE- Sepharose has been shown in Fig. 2. This step was done in order to separate Pho 2 from Pho 1, since a gradient consisting of 20-60 mM citrate can elute only Pho 2 [25] hence the peak of enzyme activity obtained corresponded only to Pho 2 which was also confirmed by Zymogram analysis (Fig. 3). The active fractions from the fraction no. 8-14 were pooled together. The total protein content of the active fractions was 0.2 mg protein and total activity was 3.5 units and therefore specific activity was 17.5 units/mg protein.

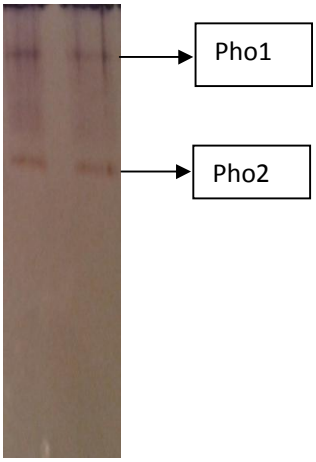

Fig. 1. Zymogram analysis. 5% polyacrylamide gel having 0.2% glycogen was used for electrophoresis and after electrophoresis; gel was incubated in the reaction mixture having glucose-1-phoshate in Tris maleate buffer. After incubation, gel was stained using Lugol's solution for detecting glycogen formed. Top band corresponded to Pho1 (pertaining to lower affinity for glycogen) and lower band corresponded to Pho2 (pertaining to higher affinity to glycogen) [12]

3.1.4 Sepharose-4B gel filtration chromatography

This step was done in order to remove the contaminating proteins in the DEAE-Sepharose fraction. A total of 15 fractions each of 2 mL volume were collected and analyzed for enzyme activity. The elution profile of the Sepharose-4B chromatography has been shown in Fig. 4. A clear peak corresponding to Pho 2 was obtained and the active fractions were pooled and concentrated. SDS-PAGE showed only one band of protein indicating the sample to be homogeneous (Fig. 5). The total protein which corresponded mainly to Pho 2 was found to be 0.02 mg and the total activity was found to be 3.0 units and therefore specific activity was 150.

3.2 Molecular Weight

The native molecular weight of Pho 2 was found to be 188,000±3000 daltons by gel filtration chromatography through Sephadex G-200 (Fig.

6). The sub-unit molecular was found to be 94,000±2000 daltons by sodium dodecyl sulfate polyacrylamide gel electrophoresis (Figs. 5 and 7). Shimomura et al. [26] reported 92000 daltons as subunit molecular weight for cytoplasmic starch phosphorylase from spinach leaves. Preiss et al. [9] reported 92,000±2800 daltons as subunit molecular weight for cytoplasmic starch phosphorylase from spinach leaves. Others have also reported subunit molecular weight of plant starch phosphorylase in the range of 100,000 daltons [27,28]. Higgins et al. [29] showed subunit molecular weight 110,000 daltons for Pho1 and 95,000 daltons for Pho2 in barley grains.

3.3 Optimum Temperature of Pho 2

Enzyme assay was carried out in the range of 10ºC to 60ºC to find out the optimum temperature. In the direction of polysaccharide synthesis, the purified Pho 2 enzyme showed optimum temperature at 37ºC and the half-maximal activity at 30ºC and 40ºC (Fig. 8).

Starch phosphorylase from sorghum leaves has been shown to have 40ºC as optimum temperature [30]. Garg and Kumar [17] showed optimum temperature 37ºC for starch phosphorylase from cabbage leaves. Garg and Kumar [31] also showed optimum temperature 37ºC for starch phosphorylase from germinating Indian millet. Hsu et al. [32] reported optimum temperature of starch phosphorylase from etiolated rice seedlings to be 45 to 50ºC which is somewhat higher temperature compared to other reports. In general, our results are consistent with other reports.

Table 1. Purification profile of starch phosphorylase multiple form Pho 2 from spinach leaves

Sample	Volume (ml)	Total protein (mg)	Total units of enzyme activity(Units)	Specific activity (Units/mg protein)	% Recovery	Fold enrichment
Crude extract	100	34	7.8	0.23	100	1
Ammonium sulphate (40-65%)	15	18	7.6	0.42	97	1.82
Desalting using Sephadex G-25 chromatography	10	9.8	7.1	0.72	92	3.1
DEAE sepharose chromatography	5	0.2	3.5	17.5	45	76
Sepharose 4B chromatography	5	.02	3.0	150	38	652

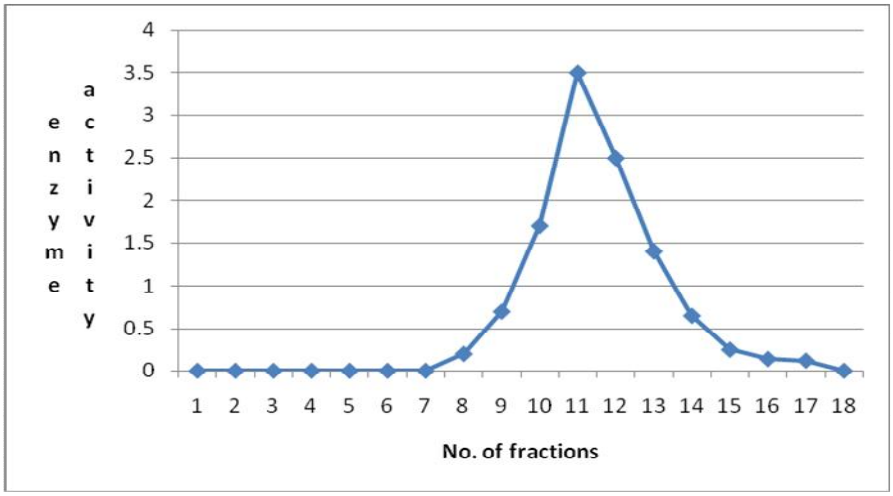

Fig. 2. Elution profile of Pho 2 on DEAE-Sepharose ion exchange chromatography

3.4 Optimum pH

In the direction of polysaccharide synthesis, enzyme assay was carried out in the range varying from pH 4.5 to pH 8 to find out the optimum pH at which the enzyme showed maximum activity. The purified Pho 2 enzyme showed optimum pH at pH 6.0 and half-maximal activity at pH 5.2 and pH 7.0 (Fig. 9).

Starch phosphorylase from tapioca leaves, cabbage leaves, Indian millet seeds has also been shown to have optimum pH 6.0 [17,31,33] Therefore, our results are consistent with other reports.

3.5 Thermal Stability

Thermal stability studies were carried out by incubating the enzyme in capped tubes in a water bath at different temperatures between 30 and 55ºC. Every 30 min, suitable aliquots were withdrawn from the tubes and stored on ice before enzyme assay. The Pho 2 enzyme had a half life of 4 h at 30ºC and 30 min at 50ºC (Fig. 10).

Starch phosphorylase from sorghum leaves, potato waste water, Indian millet and cabbage leaves has been shown to have more thermal stability at 30ºC compared to at 50ºC. It is also shown that thermal stability increased after immobilization on various solid supports [17,30,31,34].

3.6 Effect of Aromatic Amino Acids

3.6.1 Effect of tryptophan

Tryptophan was found to be inhibitory for Pho 2 enzyme activity. It showed 20% inhibition at 1 mM concentration of tryptophan whereas 79% inhibition at 5 mM concentration of tryptophan (Fig. 11). The Ki value was calculated to be 2.9 mM as determined using Dixon plot.

3.6.2 Effect of phenylalanine

Phenylalanine was also found to be inhibitory for Pho 2. It showed 18% inhibition at 1 mM concentration of phenylalanine whereas 82% inhibition at 5 mM concentration of phenylalanine (Fig. 12). The Ki value was calculated to be 2.8 mM as determined using Dixon plot.

Starch phosphorylase from sorghum leaves and from Indian millet has been shown to be inhibited by aromatic amino acids and the inhibition has been interpreted as feedback inhibition since for the biosynthesis of aromatic amino acids, phosphoenolpyruvate (PEP) and erythrose-4-phosphate are the starting substrates and these starting substrates are derived from starch via reaction by starch phosphorylase [30,31]. Soman and Philip [35] also showed inhibition of fish phosphorylase by aromatic amino acids.

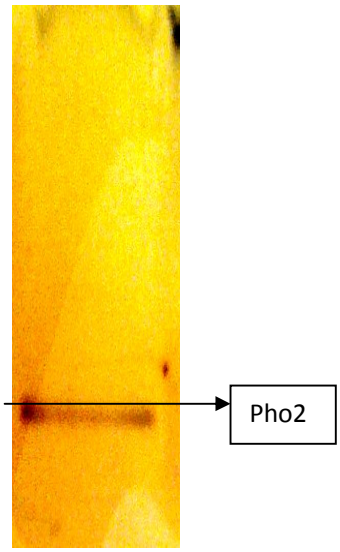

Fig. 3. Zymogram analysis. 5 % polyacrylamide gel having 0.6 % glycogen was used for electrophoresis and after electrophoresis gel was incubated in the reaction mixture having glucose-1-phoshate in Tris maleate buffer. After incubation, gel was stained using Lugol's solution for detecting glycogen formed. It showed only one band of enzyme activity which corresponded to Pho 2 (pertaining to higher affinity to glycogen)

3.6.3 Determination of K_m and V_{max} for starch and glucose-1-phosphate

The Michaelis constant (Km) of starch for Pho 2 was calculated to be 116 µg/mL using Lineweaver-Burk plot whereas Km of glucose-1-phosphate was calculated to be 0.55 mM (Figs. 13 and 14). The Km values of starch and glucose-1-phosphate for starch phosphorylase have been reported in the same range [33]. Preiss et al. [9] reported Km value of glucose-1-phosphate as 0.64 mM for Pho2.

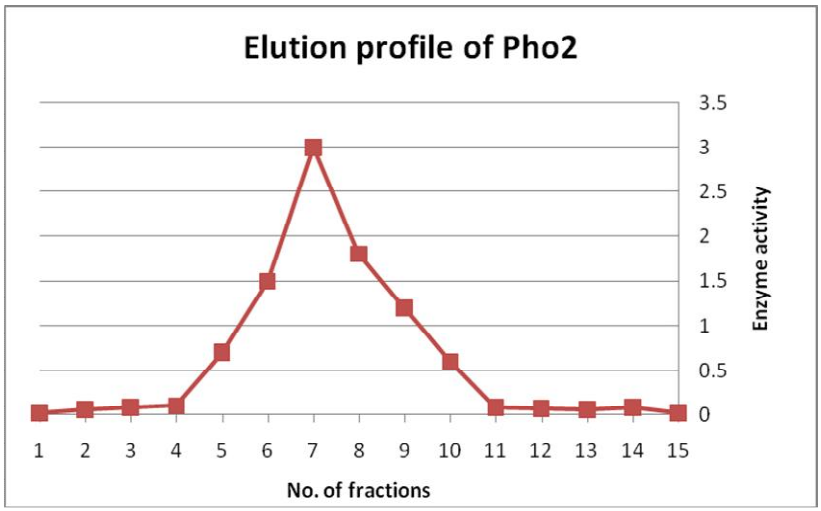

Fig. 4. Elution profile of Pho 2 on Sepharose-4B gel filtration chromatography

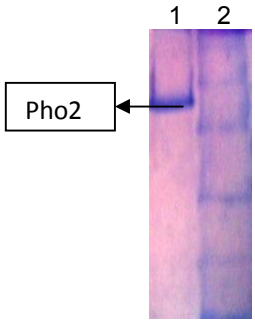

Fig. 5. SDS-PAGE of Pho 2 after Sepharose-4B chromatography as resolved on 8% polyacrylamide gel (Lane-1 Pho 2, Lane-2 standard marker 34-200 daltons)

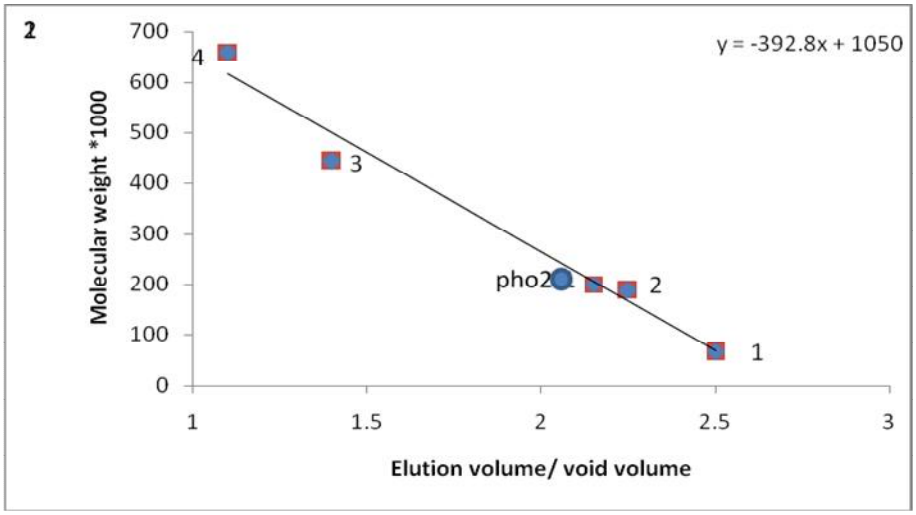

Fig. 6. Molecular weight determination of Pho2 form of starch phosphorylase using Sephadex G-200 gel filtration chromatography. 1- bovine serum albumin (MW66, 000), 2- β-amylase (MW200,000), 3-apoferritin (MW443,000), 4-thyroglobulin (MW.669.000), ● - Pho2

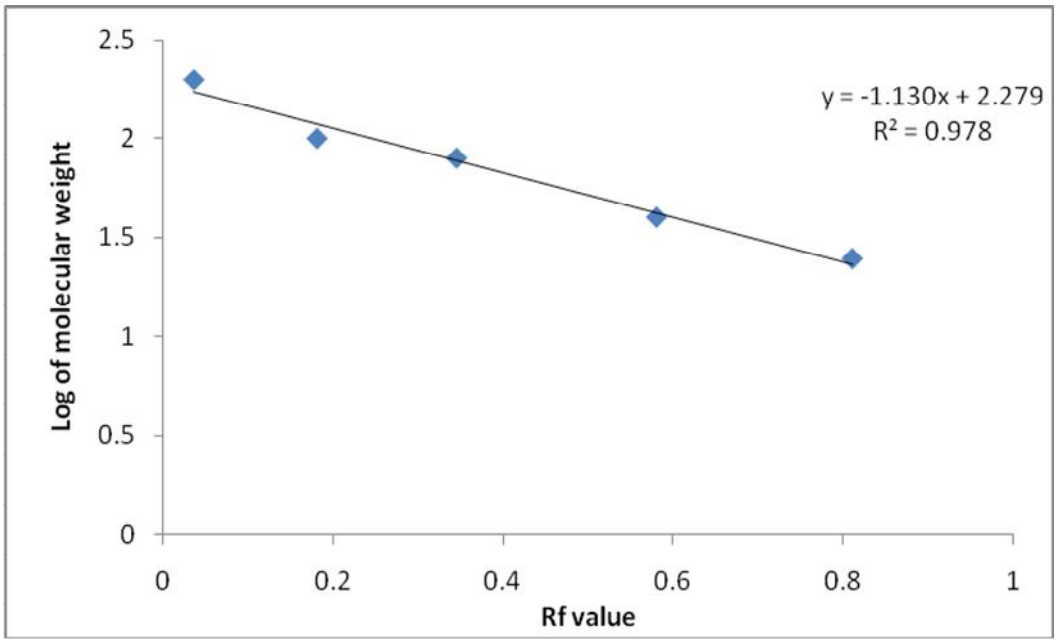

Fig. 7. Subunit molecular weight determination using SDS polyacrylamide gel electrophoresis. standard molecular markers mid range 34-200 KDa were used for comparison

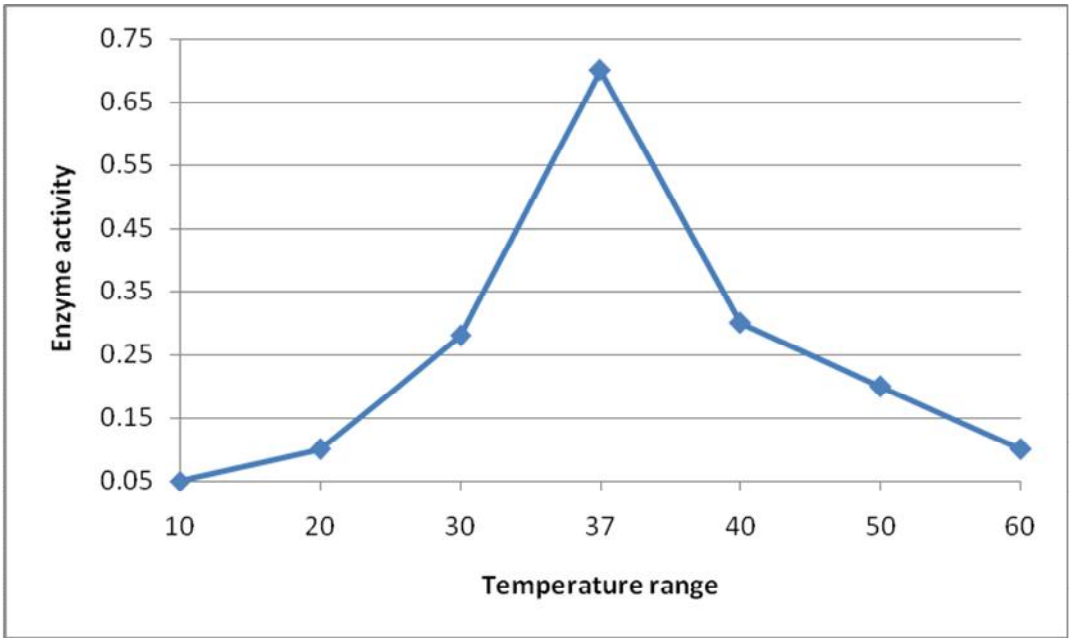

Fig. 8. Temperature- Pho 2 enzyme activity relationship. Optimum temperature has been calculated to be 37°C with half temperature maxima at 30°C and 40°C

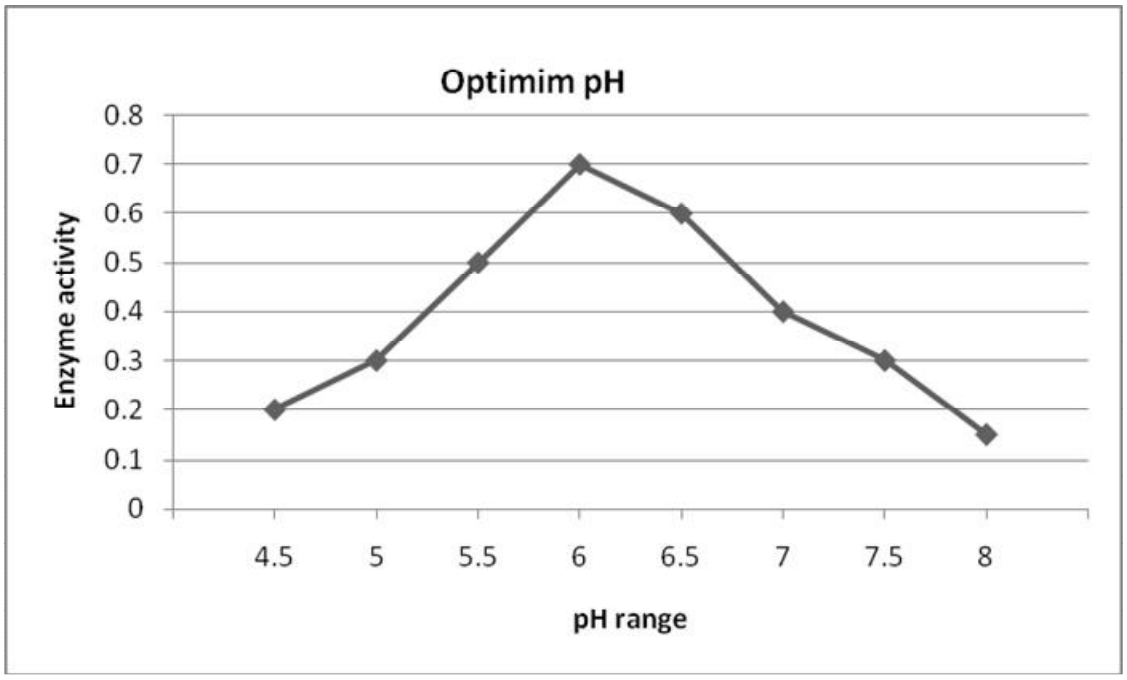

Fig. 9. pH- Pho 2 enzyme activity relationship. Optimum pH has been calculated to be pH 6.0 with half pH optima at pH 5.2 and 7.0

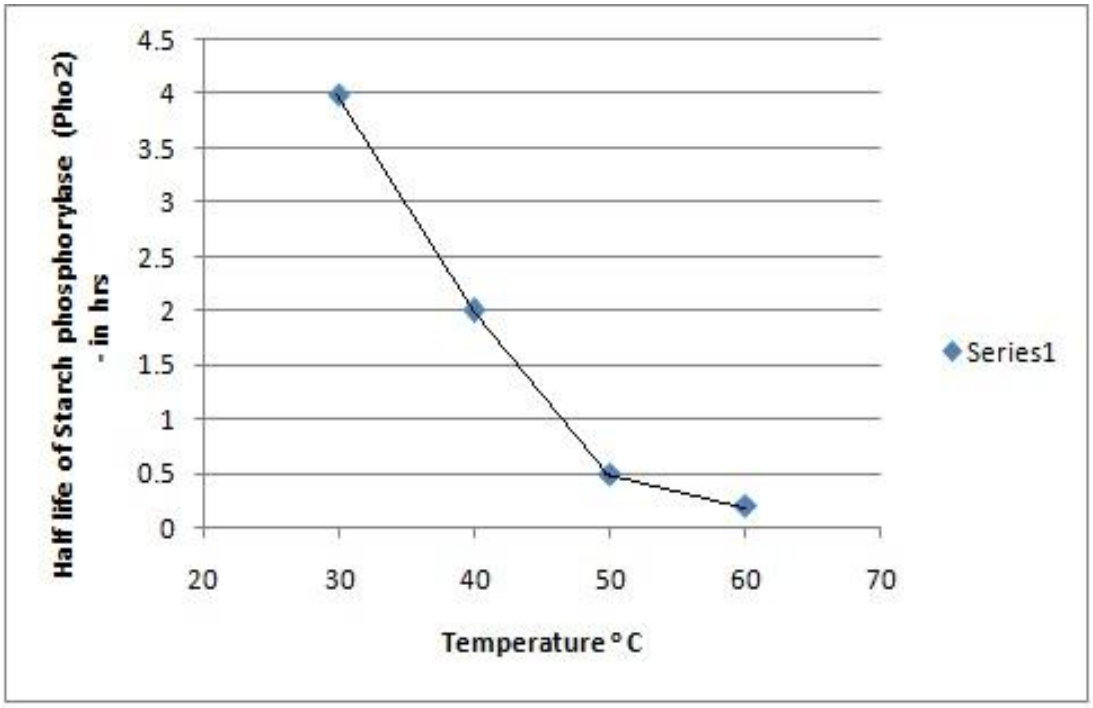

Fig. 10. Thermal stability profile of Pho2. It had half life of 4 h at 30°C and 30 min at 50°C

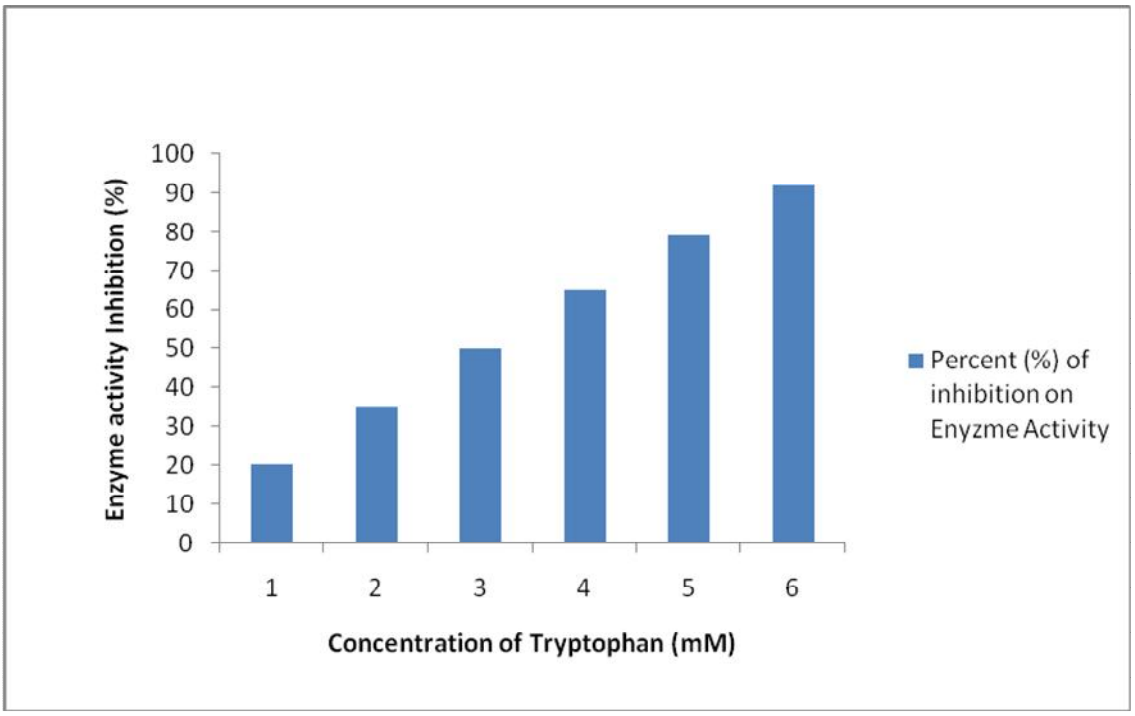

Fig. 11. Effect of tryptophan on Pho 2 enzyme activity. There was 20% inhibition by 1 mM tryptophan

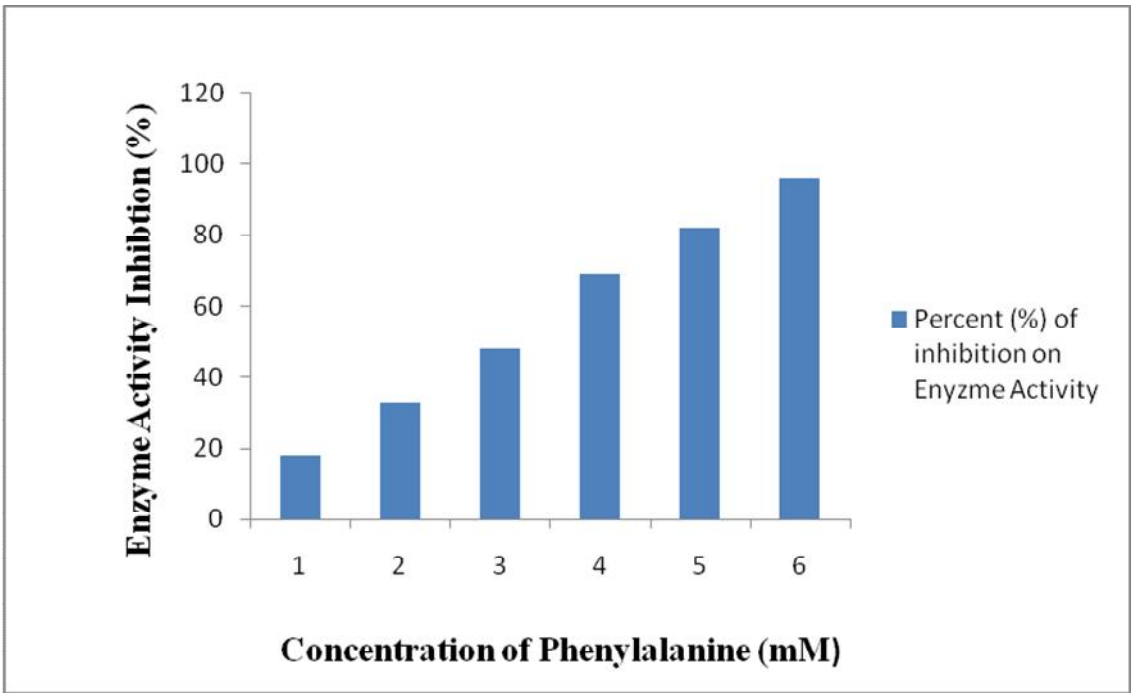

Fig. 12. Effect of phenylalanine on Pho 2 enzyme activity. There was 18% inhibition by 1 mM phenylalanine

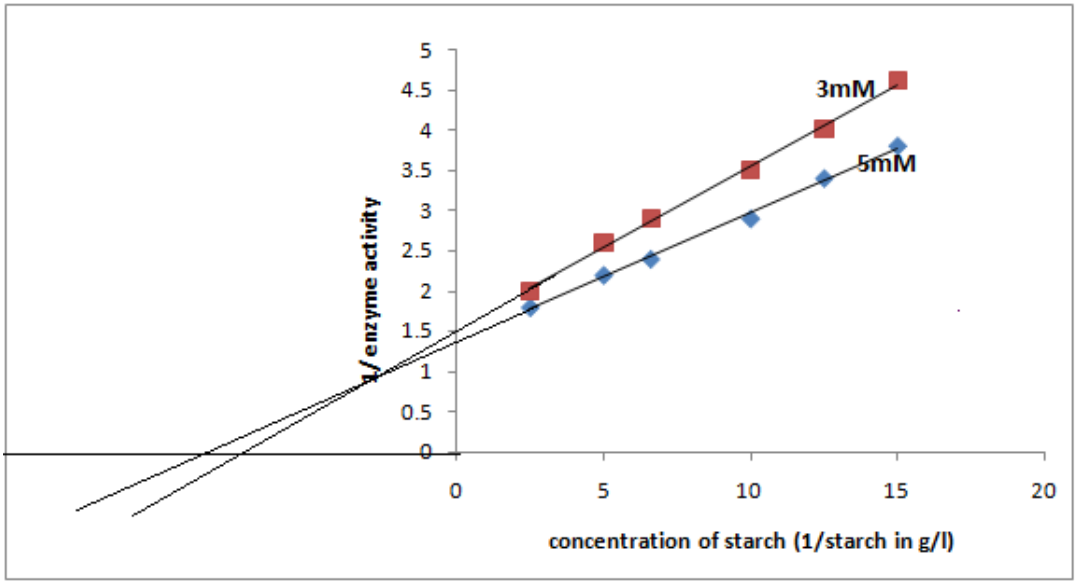

Fig. 13. Lineweaver- Burk plot: enzyme activity as a function of starch concentration in the presence of constant concentration of glucose-1-phosphate (3 mM and 5mM)

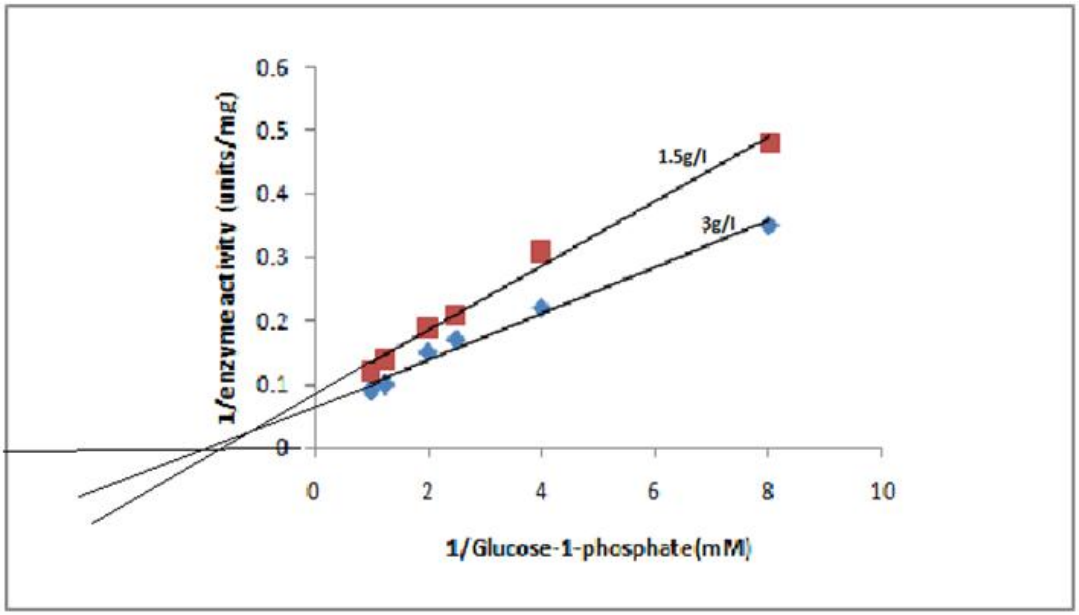

Fig. 14. Lineweaver- Burk plot: 1/enzyme activity versus 1/glucose-1-phosphate concentration plot. The Km of Pho 2 for glucose-1-phosphate has been calculated to be 0.55 mM.

4. CONCLUSION

In this article, we have purified Pho2 multiple form of α-glucan phosphorylase from spinach leaves using ammonium sulfate fractionation, DEAE-Sepharose and Sepharose-4B chromatographies. The purified enzyme showed single band on sodium dodedyl sulphate polyacrylamide having a molecular weight of 94,000±2000 daltons. The native molecular weight has been found to be 188,000±3000 as determined using Sephadex G-200 chromatography. The optimum pH of Pho2 in the direction of starch synthesis has been reported with two half pH optima. Optimum temperature with two half temperature optima has also been

reported. Thermal stability of the enzyme at different temperature has been reported. Half life of the enzyme at 30ºC and 50ºC has been reported. The Km values for glucose-1-phosphate and starch have been reported. α-Glucan phosphorylase is an important enzyme of carbohydrate metabolism and has been exploited for many industrial purposes including starch and paper industries. α-Glucan-phosphorylase plays an important role to manage abiotic stress in plants during adult growth stage of plants and seed development because at that time there is imbalance in carbohydrates levels which makes this enzyme physiologically important. In future, work may be carried out on chimeric α-glucan-phosphorylase for improving resistance in plants against abiotic stress.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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