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Partial Purification and Characterization of Minor Form of Phosphofructokinase from the Host Fraction of Chickpea (*Cicer arietinum* L. cv. Amethyst) Nodules

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Abstract : The minor form of phosphofructokinase (EC 2.7.1.11; PFK), which was suggested to be of plastid origin from the host fraction of chickpea nodules, was isolated as a small protein with apparent molecular mass near 220 kDa and purified to a high degree. SDS-PAGE and western blot indicated that the enzyme was made up of a homotetrameric structure (55 kDa). The enzyme had sharp pH profiles with maximal activities at pH 8 and displayed Michaelis-Menten kinetics with respect to Fru-6-P and nucleoside triphosphate substrate at the pH optimum (pH 8) and at pH 7. MgATP was the most effective phosphoryl donor. Phosphoenolpyruvate was a potent inhibitor of minor PFK activity, and the enzyme was also strongly inhibited by 3-phosphoglycerate, 2-phosphoglycerate, and to a lesser extent, PPi. Minor PFK was weakly activated by KCl, NaCl and Pi, and was inhibitory at high concentration of KCl and Pi.(Received July 8, 1998; accepted August 12, 1998)

Introduction

Symbiotic nitrogen fixation in leguminous root nodules is energetically costly to the host plant. Photosynthate, translocated into the nodules predominantly as sucrose, is required for nodule growth and maintenance, and to regulate the supply of carbon substrates for the bacteroids and the assimilation of fixed ammonia. These processes take place under micro-aerobic conditions which need to be maintained in the nitrogen-fixing zone of nodules to protect the nitrogenase complex from damage by O_2. Knowledge of how nodules actively metabolize carbon substrates under these microaerobic conditions is of considerable interest to further our understanding of effective nitrogen-fixing symbioses, and has been studied most extensively in soybean (*Glycine max* L.) nodules.[1,2] Much less is known of carbon metabolism in indeterminate nodules (such as chickpea), which differ from determinate nodules (such as soybean) morphologically and in the nature of nitrogenous compounds exported into the xylem.[3]

The glycolytic pathway is likely to provide the major route for the conversion of sucrose to dicarboxylic acids such as malate, which are the preferred substrates taken up by the bacteroids.[1] The phosphorylation of Fru-6-P to Fru-1,6-bisP is the first unique reaction of this pathway, and hence a strategic point for regulation. Plant tissues contain two enzymes capable of catalyzing this step: an ATP-dependent phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11; PFK), which occurs in the cytosol and plastids and catalyzes a reaction that is essentially irreversible, and a pyrophosphate-dependent phosphofructophosphotransferase (pyrophosphate-fructose-6-phosphate 1-phosphotranferase, EC 2.7.1.90) which is a cytosolic enzyme that catalyzes a readily reversible reaction.[4-6] Extensive studies of cytosolic PFK from animals and plants indicate that the enzyme has a major role in regulating the flux through the glycolytic pathway.[5-7] In general, fine control of PFK in the cytosol is likely to involve a combination of mechanisms, which may include modification of the kinetic properties in response to changes in pH, concentration of substrates, activators and inhibitors, changes in the states of aggregation, and phosphorylation/dephosphorylation of the protein.[5,6,8] Knowledge of the relative importance of these mechanisms is likely to indicate how glycolytic activity is regulated to meet the specific metabolic requirements of a tissue. In comparison, the minor form of PFK from the plastids of plants has been studied to a much lesser extent. The main goal of our current work is the purification and characterization of the minor form of PFK from the host fraction of chickpea nodules. In the present report, we describe the physical and kinetic properties of the minor form of PFK from the host fraction of chickpea nodules, and consider the possible role of the enzyme in the fine control of glycolysis in this

Key words : carbon metabolism, chickpea, *Cicer arietinum*, phosphofructokinase, root nodules.

indeterminate symbiosis.

Materials and Methods

Materials

Chickpea (*Cicer arietinum* L. cv. Amethyst) seeds were surface sterilized in 0.4% (w/v) sodium hypochlorite for 10 min, rinsed thoroughly with running tap water for 15 min, inoculated with *Rhizobium* sp. (*Cicer*) CC1192, and sown in moistened perlite in pots at a depth of approximately 2 cm and 3~4 cm apart. Plants were grown in glasshouse with average day and night temperatures of 25 and 19°C, respectively. N-free nutrient solution[9] was given to the plants every 3-4 days. Fractogel TSK-HW 55 (F) was from E. Merck, Darmstadt, Germany, and Q-Sepharose, Blue-Sepharose and Superose 6 Prep Grade from Pharmacia, Uppsala, Sweden. Other biochemicals were Sigma Chemicals Co. St Louis, MO, USA, or Boehringer-Mannheim GmbH, Mannheim, Germany.

Purification of PFK (II)

(1) Preparation of Extracts

Nodules (50 g) from 50- to 55-day-old chickpea plants were rinsed with distilled water and homogenized with a mortar and pestle in 4 volumes of an ice-cold extraction buffer A (100 mM Tris-HCl, pH 8, containing 1 mM EDTA, 5 mM $MgCl_2$, 400 mM mannitol and 5 mM 2-mercaptoethanol). The homogenate was squeezed through a single layer of Miracloth (Calbiochem, San Diego, CA, USA) and centrifuged at 25,000 g for 10 min. The supernatant, which contained the soluble contents from the host part of the nodules, was used for further protein purification. If not stated otherwise, all steps were carried out at 4°C.

(2) Ammonium Sulfate Precipitation

Solid ammonium sulfate was slowly added to the crude extract with gentle stirring, and the protein that precipitated between 25 and 50% was collected by centrifugation at 20, 000 g for 10 min. The precipitate was dissolved in buffer B (5 ml of 20 mM Tris-HCl, pH 8, containing 5 mM $MgCl_2$, 1 mM EDTA, 20 mM KCl and 5 mM 2-mercaptoethanol).

(3) Gel-Filtration Chromatography on Fractogel TSK-HW 55(F)

The protein solution applied to a Fractogel TSK-HW 55(F) column (115×2.5 cm) which had been equilibrated previously with buffer B and calibrated with blue dextran 2000 (Vo), thyroglobulin (669 kDa), ferritin (450 kDa), catalase (240 kDa), aldolase (158 kDa) and BSA (66 kDa). The chromatography was performed at room temperature (20 to 22°C) using a flow rate of 2.5 ml min^{-1}. The included fractions, which contained the second peak of PFK activity, were pooled.

(4) Anion-Exchange Chromatography on Q-Sepharose

Active fractions were applied to a Q-Sepharose column (25×2.5 cm) which had been equilibrated previously with buffer B. The column was washed with 40 ml of buffer B and PFK (II) eluted with a gradient produced by introducing 100 ml of buffer B containing 1 M KCl into 100 ml of buffer B. The flow rate was 1.5 ml min^{-1} and fractions of 4 ml were collected. Active fractions were pooled and dialyzed overnight against 2 L of 20 mM Tris-HCl (pH 8), 1 mM $MgCl_2$ and 1 mM 2-mercaptoethanol (buffer C).

(5) Affinity Chromatography on Green 19 Dye

The dialyzed protein solution (5 ml each) was divided into 0.5 ml aliquots and then applied to 10 prepacked green 19 dye columns (4.5×0.7 cm, Sigma), which had been equilibrated previously with buffer C. The columns were washed with 15 ml of buffer C and then step-eluted with NaCl at 0.2 and 1 M (15 and 25 ml, respectively), and the eluate was collected in 2 ml fractions. Fractions containing enzyme activity were pooled and dialyzed for 2 h against 2 L of buffer D (20 mM Tris-HCl, pH 8, 1 mM EDTA, 20 mM KCl, 5 mM $MgCl_2$ and 1 mM 2-mercaptoethanol), and concentrated overnight to 0.5 ml in a dialysis bag which was covered with sucrose.[10] Preparations obtained after this step contained less than 2% Fru-1,6-bisphosphatase, ATPase, NADH oxidase, phosphohexose isomerase, and phosphofructo-phosphotransferase activities, and were used for kinetic studies.

(6) Gel-Filtration Chromatography on Superose 6 Prep Grade

For further purification, the concentrated enzyme from the Green 19 dye step was chromatographed at 20 to 22°C through a Superose 6 Prep Grade column (47×1 cm) using a flow rate of 1.5 ml min^{-1} in buffer D. Active fractions were pooled, dialyzed against 2 L of buffer C for 2 h, concentrated to 1 ml in sucrose, and stored at -15°C.

Assay of enzyme activity

All enzyme assays were performed at 30°C in reaction mixtures which had a final volume of 1 ml. The activity of major or minor form of PFK was measured in a continuous assay in which the formation of Fru-1,6-bisphosphate was coupled to the oxidation of NADH in the presence of excess aldolase (EC 4.1.2.13), triose phosphate isomerase (EC 5.3.1.1) and α-glycerol phosphate dehydrogenase (EC 1.1.1.8), and the decrease in A_{340} monitored.[5] The reaction rate was linear for at least 5 to 10 min and was proportional to the amount of enzyme added. Reaction mixtures for the standard assay contained 50 mM Tris-acetate (at the pH indicated), 2 mM $MgCl_2$, 2 mM Fru-6-P, 1 mM ATP, 0.2

mM NADH, 0.4 mg BSA, 1 unit (U) aldolase, 1 U triose phosphate isomerase, 1 U α-glycerol phosphate dehydrogenase and an appropriate amount of enzyme. One U of enzyme activity is defined as the amount of enzyme that catalyzed the formation of 1 μmol of product min^{-1}. PFK and the coupling enzymes were free of low molecular mass compounds prior to use in assays with an EconoPac desalting column (Bio-Rad, Hercules, CA, USA) in 20 mM Tris-acetate. Assay mixtures were preincubated for 5 min before initiating the reaction with either of Fru-6-P or MgATP. For assays conducted during the purification procedures, 50 mM Tris-HCl (pH 8) was used instead of Tris-acetate.

Values for K_m and maximum velocity (V_{max}) were determined from initial rate measurements from at least 10 concentrations of Fru-6-P between 0.05 and 4 mM, and of the nucleoside triphophate between 5 and 200 μM. Data were first analyzed graphically to check the linearity of double reciprocal plots, and then fitted to the Michaelis-Menten equation by nonlinear regression.[11] Protein concentration was determined with Coomassie Blue reagent (Bio-Rad) according to the manufacturer's instructions, using BSA as a standard. Sucrose and 2-mercaptoethanol were removed from samples for protein determination by dialysis for at least 6 h against 2 L of 10 mM Tris-HCl (pH 8).

Electrophoresis and Western blot analysis

SDS-PAGE with gels of 10% polyacrylamide was carried out by the procedure of King and Laemmli (1971).[12] For western blot analysis, highly enriched fractions were used. As a control, a 25 to 50% ammonium sulfate precipitate of a soluble protein extract of chickpea nodules was desalted with an EconoPac desalting column and also tested with polyclonal antibodies (1:1000 dilution) against the minor form of PFK from soybean nodule.[13] The nitrocellulose membrane was blocked with 3% gelatin (Bio-Rad) and then incubated for 2 h with the primary antiserum in TBS (10 mM Tris-HCl, pH 7.5, and 150 mM NaCl) containing 1% gelatin. The blot was subsequently washed twice for 15 min in TBS containing 0.1% Tween 20 with 1% gelatin. For the detection of the bound antibodies, the blot was incubated for 2 h with alkaline phosphatase-conjugated goat anti-rabbit IgG antibody (Bio-Rad) in TBS with 1% gelatin at a dilution of 1:1000. After the blot was washed once for 15 min in TBS with 1% gelatin and twice for 15 min each in TBS containing 0.1% Tween 20 and no gelatin, the color reaction was carried out according to the manufacturer's protocol (Bio-Rad).

For the detection of N-linked glycoproteins, the nitrocellulose membrane was decorated with concanavalin A or an antiserum against Xyl-containing complex glycans, as described by Faye and Chrispeels (1985) and Laurière *et al.* (1989).[14,15]

Results

The minor form of PFK was purified to a specific activity of 125 unit/mg protein with an overall recovery of 3% from the host fraction of chickpea nodules using the procedure summarized in Table 1. Two peaks of PFK activity were observed when a 25~50% ammonium sulfate precipitation from the host part of chickpea nodules was chromatographed through a Fractogel size exclusion column. The major peak (first peak) in the excluded fraction was accounted for 70% of the PFK activity recovered, whereas the minor fraction (second peak) was recovered in approximately 30% of the PFK activity. The minor form of PFK was eluted in a single peak from the Q-Sepharose column, with maximum activity corresponding to a KCl concentration of 0.27 M, whereas the maximal activity of major form was detected at 0.4 M KCl. A single peak of the activity of minor form was also observed in subsequent chromatographic steps.

Green 19 dye affinity chromatography and Superose 6 Prep Grade gel-filtration chromatography for the minor form of PFK was the most effective procedure for the removal of contaminating proteins from the preparations. Although the recovery of enzyme was less than 3% on Superose 6, it was necessary step to obtain pure enzyme. The purified enzyme lost less than 20% of its activity when stored for 1 week at -15°C in 20 mM Tris-HCl, pH 8, 1 mM $MgCl_2$, 1 mM 2-mercaptoethanol and 50% (w/v) sucrose, but was unstable

Table 1. Partial purification of the minor form of PFK from the host fraction of chickpea nodules. Nodules (50 g) from 50- to 55-day-old plants were extracted as described

Fraction	Activity (unit)	Protein (mg)	Specific activity (unit/mg protein)	Recovery (%)	Purification (fold)
Crude extract	48.2	351	0.14	100	1
25~50% $(NH4)_2SO_4$	39.2	108	0.36	81	3
Fractogel	6.8	10.9	0.62	14	5
Q-Sepharose	5.6	0.9	6.3	11	45
Green 19 dye	4.1	0.1	41	8	293
Superose 6	1.5	0.012	125	3	892

when sucrose was replaced by 50% (v/v) glycerol, 1 mM ATP, 5 mM dithiothreitol, 5 mM Fru-6-P or 30 mM KCl.

The native molecular mass of the minor form of PFK, as determined by gel filtration through Fractogel TSK-HW 55(F) and Superose 6 Prep Grade, was 221±5 kDa (means ±SE of 5 determinations). Three polypeptide bands, with molecular mass of 72±1 kDa, 70±1 kDa and 55±1 kDa (means±SE of 5 determinations), were stained with Coomassie Blue reagent when the most highly purified preparations of the minor form of PFK were subjected to SDS-PAGE (Fig. 1A). To determine the immunological relationship between polypeptides of plastid PFK from the host fraction of soybean nodules and polypeptides of PFK from the host fraction of chickpea nodules, a polyclonal antibody against plastid PFK from soybean nodule recognized only one polypeptide (55 kDa) in an ammonium sulfate precipitate of 25 to 50% saturation (Fig. 1B), but did not show cross-reaction with the major form of PFK from plant fraction of chickpea nodules (data not shown). Furthermore, to test whether the partially purified PFK were N-linked glycoproteins, they were decorated on western blots with concanavalin A or an antibody against Xyl-containing complex N-linked glycans from plants. Neither the lectin nor the anti-carbohydrate antibody bound to the proteins (data not shown).

In a series of Tris-acetate, imidazole-acetate and triethanolamine-acetate buffers, the purified enzyme had sharp pH profiles with maximum activity at pH 8 and activities of 80% or more of the maximum between pH 7.5 and 8.5 (Fig. 2). Studies of the kinetic properties were performed at the optimum pH (pH 8) and at a pH nearer to the physiological value (pH 7), where activity was approximately 60% of the maximum. The variation of three buffers was not observed with the minor form of PFK.

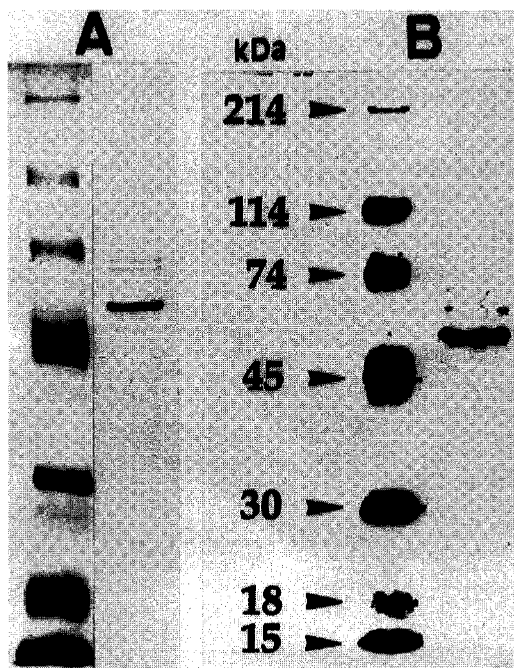

Fig. 1. SDS-PAGE (A) and western blot (B) of the minor form of PFK from the host fraction of chickpea nodules. Electrophoresis was performed in a 10% gel according to the method of King and Laemmli (1971). The sample applied to the lanes was: 2 μg of Superose 6 fraction (A) and 10 μg of 25∼50% $(NH_4)_2SO_4$ fraction (B). Same molecular mass standards were used in (A) and (B).

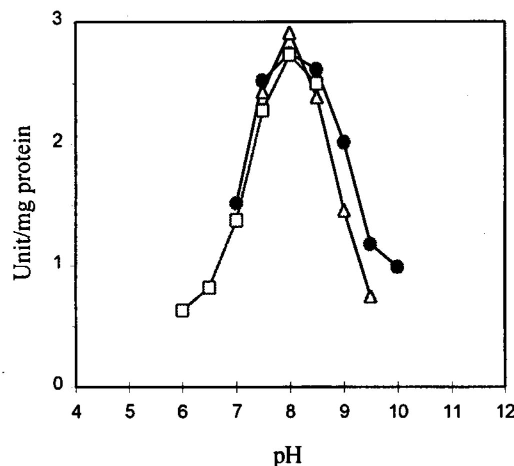

Fig. 2. Effect of pH on activity of minor form of PFK. Reaction mixtures were as described for standard assay except that Tris-acetate (●), imidazole-acetate (□) and triethanolamine-acetate (△) were used at the pH values indicated.

The enzyme displayed typical hyperbolic kinetics when MgATP and Fru-6-P were the varied substrates at pH 7 and pH 8. Data from initial velocity experiments with both substrates gave linear double reciprocal plots and fitted well to the Michaelis-Menten equation. With ATP and $MgCl_2$ at concentration of 1 mM and 5 mM, respectively, the K_m values for Fru-6-P were 0.99±0.06 mM at pH 7 and 1.1± 0.09 mM at pH 8 (Table 2). The K_m values for MgATP were approximately 33.1 μM and 23.2 μM at pH 7 and pH 8, respectively. MgCTP, MgGTP, MgITP and MgUTP were also able to act as the phosphoryl donor; the K_m values with these nucleoside phosphates were 1.2 to 1.8 fold higher than for MgATP, and V_{max}/K_m was between 51∼87% of that with MgATP (Table 2).

The enzyme had no activity when $MgCl_2$ was omitted from the reaction mixtures at pH 7 and pH 8. In the presence of 1 mM ATP, activity was maximal with 5 mM $MgCl_2$ at pH 7 and 8 (Fig. 3A). An excess of $MgCl_2$ over ATP caused only slight inhibition, whereas an excess of ATP over $MgCl_2$ was strongly inhibitory. The activity of minor form of PFK was stimulated a maximum of 1.7-fold at pH 7 and 1.4-fold at pH 8 by KCl (and NaCl) at a concentration of 30 mM, but increasing the concentration of KCl above this level led to a decline in activity (Fig. 3B). P_i at 4 mM stimulated the activity 1.4-fold at pH 7 and pH 8

Table 2. Kinetic parameters of the minor form of PFK from the host fraction of chickpea nodules. Reaction mixtures were as for the standard assay. An excess of 5 mM $MgCl_2$ was maintained over the concentration of the nucleoside triphosphate. K_m is in μM and Vmax in unit/mg protein. The values are the means±SE of 3 replicate experiments

Substrate	pH 7		pH 8	
	K_m	V_{max}/K_m	K_m	V_{max}/K_m
MgATP	32.1±0.8	70±2	23.2±0.4	134±5
MgCTP	39.1±0.9	59±5	30.1±0.2	103±4
MgGTP	44.5±1.2	51±7	41.3±1.8	69±12
MgITP	40.1±0.5	58±5	32.7±0.4	95±5
MgUTP	38.2±1.1	61±4	28.6±0.5	99±7
F-6-P	990±60	2.2±0.2	1,093±90	3.1±0.2

SE: Standard error of the mean.

(Fig. 3C). Higher concentrations of P_i were inhibitory.

Activity of minor form of PFK was influenced by several metabolites. Phosphoenolpyruvate was the most potent inhibitor, with concentrations of less than 10 μM inhibiting activity by 50% at pH 7 and pH 8 (Table 3). The enzyme was also strongly inhibited by 2-phosphoglycerate, with the $I_{0.5}$ value of 9.8 μM at pH 7 and 36 μM at pH 8, and to a lesser degree by 3-phosphoglycerate and pyrophosphate. Inhibition by these metabolites was more sensitive at pH 7 than pH 8.

Discussion

The partially purified enzyme from the host fraction of chickpea nodules corresponded to a protein with an apparent native molecular mass of 220 kDa. It is likely that the enzyme was from host fraction of the nodules, since PFK activity was not detected in the bacteroids of chickpea nodules.[16] A similar pattern of the minor form of PFK was found in carrot root and soybean nodule,[5,17] which had molecular mass of approximately 400 and 200 kDa, respectively, but, in contrast, the minor form of PFK was not found in tomato fruits.[18] To detect the disaggregated state of the partially purified chickpea nodule PFK by MgATP, the enzyme was not disaggregated on exposing state of 10 mM MgATP in contrast to the major form of PFK from other plant tissues.[5,6,17] On purification of the minor form of PFK, comparable specific activities were reported for other highly purified preparations of the minor form of PFK from plants, including the host fraction of potato tuber and soybean nodules.[13,19]

Highly purified preparations of the minor form were resolved by SDS-PAGE into three polypeptides, but it was not possible to conclude that the enzyme was to be a heteropolymer. In relationship between native molecular mass and subunits of the minor form, it might be suggested

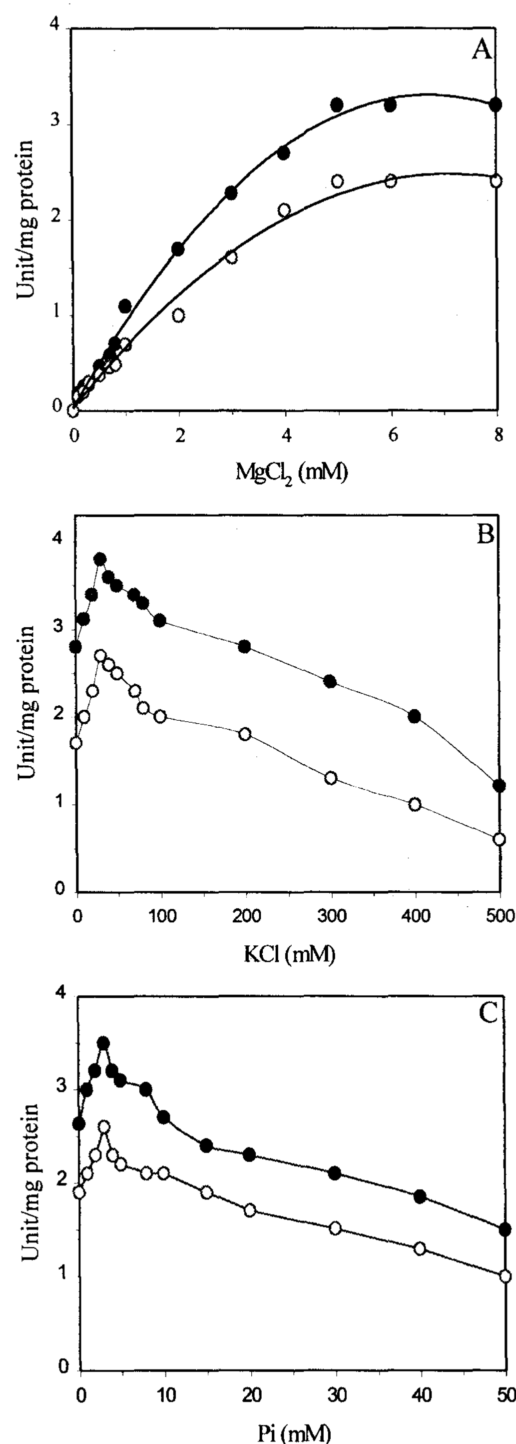

Fig. 3. Effect of $MgCl_2$, KCl and Pi on activity of the minor form of PFK. Reaction mixtures were of the composition described for the standard assay at pH 7 (○) and pH 8 (●).

that the minor form of PFK is a homotetramer with 55 kDa subunit, but it was not possible to be native molecular mass with three subunits (72, 70 and 55 kDa) or only two subunits (72 and 70 kDa). Furthermore, in regard to the result of the western blot, it could also support that the minor form of PFK had a tetrameric structure with 55 kDa subunits. Highly purified PFK from castor bean leucoplasts

Table 3. Effect of inhibitors on the minor form of PFK from the host fraction of chickpea nodules. Reaction mixtures were of the composition described for the standard assay. Concentrations that inhibited PFK by 50% ($I_{0.5}$) are in μM and are the means±SE from 3 replicate experiments

Inhibitor	$I_{0.5}$ (μM)	
	pH 7	pH 8
Phosphoenolpyruvate	4.0±0.1	9.1±0.1
2-Phosphoglycerate	9.8±0.2	36±2
3-Phosphoglycerate	5.0±0.1	300±17
Pyrophosphate	190±12	2,200±189

and *Selenastrum minutum* contained only a single polypeptide.[20,21] In contrast, the multiple polypeptides were reported to be present from cucumber seeds and soybean nodules.[5,22]

In the immunological studies, the polyclonal antibody of the minor form of PFK from soybean nodules were specific for the minor form of PFK from chickpea nodules and did not cross-react with the major form of PFK from chickpea and soybean nodules. Unlike the major form of PFK from the host fraction of chickpea nodules and soybean nodules, these results strongly supported the hypothesis that the minor form of PFK from chickpea nodules had a molecular similarity with the minor form of PFK from soybean nodules.[13] Furthermore, in studying N-linked glycoprotein of the partially purified PFK, similar results were obtained with the minor form of PFK from castor oil seed and soybean nodule, which supported the proposal that the enzyme was located in the cytoplasm or plastid.[5,23]

The minor form of PFK displayed typical Michaelis-Menten kinetics with respect to the nucleoside triphosphate substrate at pH 7 and pH 8. The enzyme exhibited broad specificity towards the nucleoside triphosphate substrate, although comparison of the values of the parameter V_{max}/K_m, which gives a measure of the rate of interaction of an enzyme with a substrate at low concentrations, indicated that MgATP was the most effective phosphoryl donor. The K_m for MgATP was within the range of values (10~35 μM) generally reported for PFKs from other plant sources.[5,20,22] Broad specificity for the nucleoside triphosphate substrates appeared to be a property of PFK from plants, animals and bacteria. Unlike cytosolic PFK from the developing endosperm of *Ricinus communis*, the enzyme was inhibited by increased concentrations of ATP, and was similar to the plastid PFK from soybean nodules and the developing endosperm of *R. communis.*[13,20]

Under the conditions used in this study, hyperbolic kinetics were also observed when Fru-6-P was the varied substrate at pH 7 and pH 8. The minor form of PFK from host fraction of chickpea nodules was similar to the enzyme

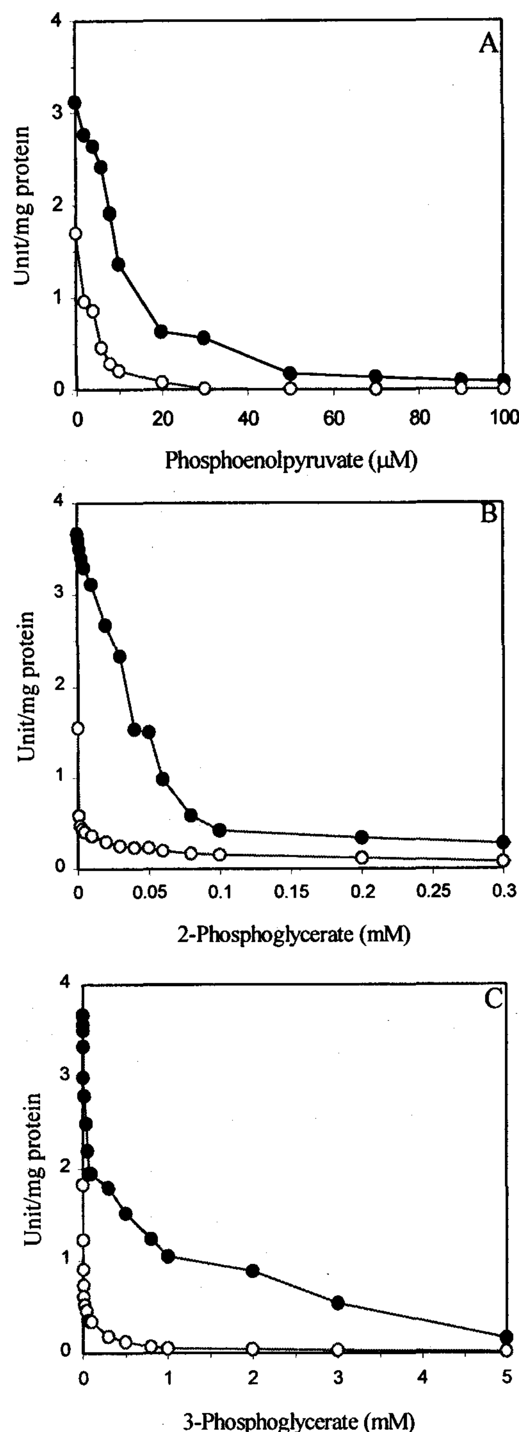

Fig. 4. Effect of phosphoenolpyruvate, 2-phosphoglycerate and 3-phosphoglycerate on activity of the minor form of PFK. Reaction mixtures were of the composition described for the standard assay at pH 7 (○) and pH 8 (●), with metabolite added as indicated.

from the cytosol of developing castor bean leaves and *Phaseolis vulgaris* seeds,[20,24] as well as PFK from cucumber seeds and tomato fruits.[18,22] In contrast, the kinetics for the interaction of Fru-6-P with PFK from other plants have been reported to be affected by pH, the ratio of Mg^{2+} to

ATP, and inhibitors and activators. In general, saturation curves for Fru-6-P with these enzymes tend to be sigmoidal in the presence of inhibitors and hyperbolic in the presence of activators.[8,21)]

Activity of the minor form was weakly stimulated by KCl (NaCl) and Pi and was inhibitory at high concentration of KCl and Pi. In this regard, the partially purified enzyme was similar to the plastid PFK from a number of plant tissues, as well as the enzyme from soybean nodules and animals.[5,7)] In contrast, KCl and Pi appeared to have much more of a stimulatory effect on the major form of PFK from cytoplasm[6)] and might not inhibit activity at high concentration. Like PFK from other plants, the minor form of PFK from chickpea nodules was not affected by Fru-2,6-bisphosphate, which was the most potent activator of PFK from mammalian tissues.[25)]

Inhibition by phosphoenolpyruvate and 3-phosphoglycerate would coordinate the activity of the minor form and other enzymes of glycolysis in chickpea nodules. Copeland *et al.* (1995)[16)] suggested that regulation of phosphoenolpyruvate carboxylase in the host fraction of chickpea nodules was important in controlling the supply of carbon substrates for the bacteroids and the assimilation of fixed ammonia. Thus, when a demand for carbon substrates was reduced, inhibition of phosphoenolpyruvate carboxylase by malate, 3-phosphoglycerate, and 2-phosphoglycerate would decrease the rate of formation of oxaloacetate from phosphoenolpyruvate. An increase in the concentration of phosphoenolpyruvate, and possibly 3-phosphoglycerate and 2-phosphoglycerate, under these conditions could reduce activity of the minor form of PFK, and, in turn, the flux through the glycolytic pathway.

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References

1. Day, D. T. and Copeland, L. (1991) Carbon metabolism and compartmentation in nitrogen-fixing legume nodules. *Plant Physiol. Biochem.* **29**, 185-201.
2. Gordon A. J. (1992) Carbon metabolism in the legume nodule. In *Carbon Partitioning: Within and Between Organisms* (Pollock, C. J., Farrar, J. F. and Gordon, A. J. eds), pp 132-166. Bios, Oxford.
3. Streeter, J. G. (1991) Transport and metabolism of carbon in legume nodules. *Adv. Bot. Res.* **18**, 129-187.
4. Salminen, S. O. and Streeter, J. G. (1987) Uptake and metabolism of carbohydrate by *Bradyrhizobium japonicum* bacteroids. *Plant Physiol.* **83**, 535-540.
5. Vella, J. and Copeland, L. (1993) Phosphofructokinase from the host fraction of soybean. *J. Plant Physiol.* **141**, 398-404.
6. Lee, H. S. and Copeland, L. (1996) Phosphofructokinase from the host fraction of chickpea nodules. *Physiol. Plant* **96**, 607-614.
7. Uyeda, K. (1979) Phosphofructokinase. *Adv. Enzymol. Rel. Areas Mol. Biol.* **48**, 193-244.
8. Copeland, L. and Turner, J. F. (1987) The regulation of glycolysis and the pentose phosphate pathway. In *The Biochemistry of Plant* (Stumpf, P. K. and Conn, E. E. eds). vol. **11**, pp. 107-128, Academic Press, New York.
9. Evans, H. Koch, J. B and Klucas, K (1972) Preparation of nitrogenase from nodules and separation into components. *Methods in Enzymology* **24**, 470-476.
10. Wolosiuk, R. A., Crawford, N. A. Yee, B. C. and Buchanan, B. B. (1979) Isolation of three thioredoxins from spinach leaves. *J. Biol. Chem.* **254**, 1627-1632.
11. Duggleby, R. G., (1984) Regression analysis of nonlinear Arrhenius plots: an empirical model and a computer program. *Comp. Biol. Med.* **14**, 447-455.
12. King, J. and Laemmli, U. K. (1971) Polypeptides of the tail fibres of bacteriophage T_4. *J. Mol. Biol.* **62**, 465-477.
13. Vella, J. (1989) Enzymes of Carbohydrate Metabolism in Soybean Nodules. Ph. D. thesis, Sydney University, Sydney, Australia.
14. Faye, L. and Chrispeels, M. J., (1985) Characterization of N-linked oligosaccharides by affinoblotting with concanavalin A-peroxidase and treatment of the bolts with glycosidases. *Anal Biochem.* **149**, 218-224.
15. Lauri re, M., Lauri re, C., Chrispeels, M. J., Johnson, K. D. and Sturm, A. (1989) Characterization of a xylose-specific antiserum that reacts with the complex asparagine-linked glycans of extracellular and vacuolar glycoproteins. *Plant Physiol.* **90**, 1182-1188.
16. Copeland, L., Lee, H. S. and Cowlishaw, N. (1995) Carbon metabolism in chickpea nodules. *Soil Biol. Biochem. vol.* **27**, 381-385.
17. Wong, J. H., Yee, B. C. and Buchanan, B. B. (1989) A novel type of phosphofructokinase from plants. *J. Biol. Chem.* **262**, 3185-3195.
18. Isaac, J. E. and Rhodes, M. J. C. (1982) Purification and properties of phosphofructokinase from fruits of *Lycopersicon esculentum. Phytochemistry* **21**, 1553-1556.
19. Kruger, N. J., Hammond, J. B. W. and Burell, M. M. (1988) Molecular characterization of four forms of phosphofructokinase purified from potato tuber. *Arch. Biochem. Biophys.* **267**, 690-700.
20. Knowles, V. L., Greyson, M. F. and Dennis, D. T. (1990) Characterization of ATP-dependent fructose 6-phosphate 1-phosphotransferase isozymes from leaf and endosperm tissues of *Ricinus communis. Plant Physiol.* **92**, 155-159.
21. Botha, F. C. and Turpin, D. H. (1990) Molecular, kinetic, and immunological properties of the 6-phosphofructokinase from the green alga *Selenastrum minutum. Plant Physiol.* **93**, 871-879.

22. Cawood, M. E., Botha, F. C. and Small, G. C. (1988) Molecular properties of the ATP:D-fructose-6-phosphate 1-phosphotransferase isoenzymes from *Cucumis sativus*. *Plant Cell Physiol.* **29**, 195-199.
23. Simcox, P. D., Reid, E. E. Canvin, D. T. and Dennis, D. T. (1977) Enzymes of the glycolytic and pentose phosphate pathways in proplastids from the developing endosperm of *Ricinus communis* L. *Plant Physiol.* **59**, 1128-1132.
24. Botha, F. C. and Small, G. C. (1987) Comparison of the activities and some properties of pyrophosphate and ATP dependent fructose-6-phosphate 1-phosphotransferases of *Phaseolus vulgaris* seeds. *Plant Physiol.* **83**, 772-777.
25. Hers, H. G. and Hue, L. (1983) Gluconeogenesis and related aspects of glycolysis. *Annu. Rev. Biochem.* **52**, 617-653.

병아리콩(*Cicer arietinum* L. cv. Amethyst) 근류내의 플라스티드 포스포프룩토오스 키나아제의 분리 및 특성

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초 록 : 병아리콩 근류의 호스트부분에서 플라스티드에 존재하는 것으로 추정되는 포스포프룩토오스 키나아제 (EC 2.7.1.11; PFK)을 순수분리 정제하고, 정제된 단백질 분자량이 220 kDa인 비당단백질(N-linked)이다. SDS-PAGE와 Western blot의 결과는 정제된 효소가 4개의 55 kDa subunit로 이루어져 있음을 지적하고 있다. 이 효소는 pH 8에서 최적활성으로 날카로운 곡선을 나타내고 있으며, 최적 pH 8과 생리적으로 비슷한 pH 7에서 Fru-6-P 및 nucleoside triphosphate 기질에 Michaelis-Menten kinetics을 나타냈다. MgATP가 뉴크레오 삼인산중에 가장 효율적인 인산기 공여체로서 나타냈다. 포스포엔롤피루베이트는 마이너 형태의 PFK 활성에 가장 강력한 억제자이며, 또한 이 효소는 3-포스포글리세레이트와 2-포스포글리세레이트에 의해 강하게 억제된다. 마이너 형태의 PFK는 KCl, NaCl등 Pi에 의해 약하게 활성이 증진되지만, 높은 농도에서는 억제자로서 작용한다.

찾는말 : 탄수화물 대사, 병아리콩, *Cicer arietinum*, 포스포프룩토오스 키나아제, 뿌리근류.