Myo-inositol content in pteridophytes and the isolation and characterization of L-*myo*-inositol-1-phosphate synthase from *Diplopterygium glaucum*

D. R. Chhetri[1]*, A. K. Mukherjee[2] and J. Adhikari[3]

*[1]Plant Physiology and Biochemistry Laboratory, Post Graduate Department of Botany, Darjeeling Government College, Darjeeling-734 101, WB, India; [2]Department of Biotechnology, Bengal College of Engineering and Technology Bidhan Nagar, Durgapur-713 212, WB, India; [3]Biochemistry Laboratory, Department of Botany, Presidency College, College Street, Kolkata, WB, India; *Corresponding author:* munal@sancharnet.in

Received: 15/03/2006, Accepted: 28/05/2006

Myo-inositol is involved in normal growth and development of all living organisms and L-*myo*-inositol-1-phosphate synthase (MIPS; EC: 5.5.1.4) is responsible for its *de novo* synthesis. This enzyme has been reported for a number of life forms including plants, animals and bacteria. In the present study free *myo*-inositol has been detected in the common pteridophytes found in the Darjeeling Himalayas and the enzyme, L-*myo*-inositol-1-phosphate synthase has been partially purified from *Diplopterygium glaucum* (Thunb.) Nakai. A crude homogenate from the reproductive pinnules of *D. glaucum* was subjected to streptomycin sulphate precipitation and 0-70% ammonium sulphate fractionation followed by successive chromatography through DEAE-cellulose, Hexylagarose and BioGel A-0.5m columns. This resulted in a partial purification of the enzyme of about 81-fold with 13.5% recovery. The pteridophytic MIPS specifically utilized D-glucose-6-phosphte and NAD^+ as its substrate and co-factor, respectively. It shows a pH optimum between 7.0 and 7.5 while the temperature maximum was 30 °C. The enzyme activity was stimulated by NH_4^+, slightly inhibited by Na^+, Ba^{2+} and Cd^{2+}, and strongly inhibited by Li^+, Zn^{2+} and Hg^{2+}. EDTA, pCMB and some substrate isomers like glucose-1-phosphate, fructose-6-phosphte and galactose-6-phosphate were inhibitory to the enzyme. The apparent molecular weight of the native *D. glaucum* MIPS was determined to be approximately 171 kDa.
Key words: D-glucose-6-phosphate, Gleicheniaceae, inositol synthase, *myo*-inositol.

Conteúdo de m*yo*-inositol em pteridófitas e o isolamento e caracterização da sintase de L-*myo*-inositol-1-fosfato de *Diplopterygium glaucum*: *Myo*-inositol está envolvido no crescimento e desenvolvimento de todos os organismos vivos e a enzima sintase do L-*myo*-inositol-1-fosfato (MIPS; EC: 5.5.1.4) é responsável pela sua síntese *de novo.* Esta enzima tem sido relatada em um número grande de plantas, animais e bactérias. No presente estudo *myo*-inositol não complexado foi detectado em pteridófitas comuns encontradas em Darjeeling Himalayas e a enzima sintase do L-*myo*-inositol-1-fosfato foi parcialmente purificada a partir de *Diplopterygium glaucum* (Thunb.) Nakai. Um extrato protéico não purificado de pinulas reprodutivas foi precipitada com sulfato de estreptomicina e 0-70% sulfato de amônia seguido de sucessivas cromatografias em colunas de DEAE-celulose, Hexylagarose e BioGel A. Este procedimento resultou na purificação parcial de 81 vezes, com recuperação de 13.5%. A MIPS dessa pteridófita usou especificamente D-glicose-6-fosfato and NAD^+ como substrato e cofator, respectivamente. Mostrou pH ótimo entre 7,0 e 7,5, enquanto a temperatura ótima foi 30°C. A atividade da enzima foi estimulada por NH_4^+, pouco inibida por Na^+, Ba^{2+} e Cd^{2+}, e fortemente inibida por Li^+, Zn^{2+} e Hg^{2+}. EDTA, pCMB e alguns substratos isômeros, como glicose-1-fosfato, frutose-6-fosfato e galactose-6-fosfato inibiram a enzima. A massa molecular aparente da MIPS de *D. glaucum* é aproximadamente 171 kDa.
Palavras-chave: Gleicheniaceae, D-glicose-6-fosfato, inositol synthase, *myo*-inositol.

INTRODUCTION

Myo-inositol is a central component of several biochemical pathways. Inositol metabolism is essential for the development of plants, animals and some microorganisms. The essential role of inositol in many cellular processes including membrane formation, cell wall biogenesis, stress response and signal transduction have been well documented (Lackey et al., 2003). *Myo*-inositol is the precursor of all inositol-containing compounds including phosphoinositides and inositol phosphates. It is formed by the conversion of D-Glucose-6-Phosphate (G-6-P) to L-*myo*-inositol-1-phosphate (I-1-P) by the enzyme L-*myo*-inositol-1-phosphate synthase (MIPS; EC: 5.5.1.4) and is subsequently dephosphorylated by inositol monophosphatase (EC: 3.1.3.25) to *myo*-inositol (Lohia et al., 1999). The MIPS reaction has been reported for archea (Chen et al., 2000), bacteria (Bachhawat and Mande, 1999; 2000), protozoa (Lohia et al., 1999), lower plants (Donahue and Henry, 1981a; 1981b; Escamilla et al., 1982; Dasgupta et al., 1984; RayChoudhury et al., 1997; Benaroya et al., 2004; Chhetri et al., 2005), higher plants (Loewus and Loewus, 1971; Loewus et al., 1978; Ogunyemi et al., 1978; Adhikari and Majumder, 1983; Johnson and Sussex, 1995; Johnson and Wang, 1996; Chun et al., 2003) and animals (Pittner and Hoffmann-Ostenhof, 1976; 1979; Maeda and Eisenberg Jr., 1980; Adhikari and Majumder, 1988; Chiu et al., 2003). The present study reports the occurrences of free *myo*-inositol in different pteridophytes. Partial purification of MIPS and characterization of the partially purified enzyme is reported here for *Diplopterygium glaucum* (Thunb.) Nakai (Family-Gleicheniaceae).

MATERIAL AND METHODS

Plant material: Fresh specimens of pteridophytes, *Dicranopteris linearis* Bedd., *Diplazium dilatatum* Bl., *Diplopterygium glaucum* (Thunb.) Nakai, *Equisetum debile* Roxb., *Lycopodium cernuum* L., *Polypodium quercifolium* L., *Pteridium aquilinum* (L.) Kuhn. and *Selaginella megaphylla* Bak. were collected from their natural habitats in and around the Darjeeling hills (ca 2134 m asl) in the Eastern Himalayas. Each experiment was carried out using three replicates under similar conditions.

Extraction and purification of MIPS: Reproductive pinnules (100 g) of *Diplopterygium glaucum* (*D. glaucum*) were collected fresh in the morning, washed twice with cold, sterile distilled water and homogenized in a chilled mortar and pestle in two volumes of 50 mM tris-acetate buffer (pH 7.5) containing 0.2 mM of ME. The crude homogenate was passed through four layers of muslin and the filtrate centrifuged at 1,000 g_n for 5 min. The supernatant was again centrifuged at 11,400 g_n for 20 min and the pellet discarded. The supernatant was dialyzed overnight against 50 mM tris-acetate (pH 7.5) buffer containing 0.2 mM ME and the clear supernatant was collected from the dialysis bag. The 11,400 g_n clear supernatant obtained from the reproductive pinnules of *D. glaucum* was subjected to streptomycin sulphate treatment to a final concentration of 2% (w/v) with constant stirring. The mixture was kept at 0ºC (in ice) for 15 min and then centrifuged at 11,400 g_n for 15 min. The supernatant (streptomycin sulphate-treated fraction) was collected and fractionated with 0-70 % $(NH_4)_2SO_4$. The precipitated protein fraction was dialyzed and this fraction (0-70 % ammonium sulphate fraction) was adsorbed onto DEAE-cellulose and the mixture packed in a glass column. The adsorbed proteins were eluted from the column with a linear gradient of 0-0.5 M KCl (figure 1A). The active DEAE-cellulose fractions (DEAE-cellulose fraction) were chromatographed on a Hexylagarose column and eluted with 50 mM Imidazole-HCl (pH 7.5) buffer containing 0.2 mM ME (figure 1B). The active Hexylagarose fractions (Hexylagarose fraction) were pooled and loaded onto a column of BioGel A-0.5m and the proteins eluted with the extraction buffer (figure 1C). The active fractions (BioGel fraction) were pooled and concentrated before further use.

Enzyme activity assay: The MIPS activity was assayed by the procedure of Barnett et al. (1970) with slight modifications (Adhikari et al., 1987). The enzyme assay mixture contained 50 mM tris-acetate (pH 7.5), 14 mM NH_4Cl, 0.8 mM NAD^+, 5 mM ME, 5 mM G-6-P and an appropriate aliquot (100-200 µg) of enzyme protein in a total volume of 0.5 mL. The mixture was incubated at 37ºC for 1h after which the reaction was terminated by adding 0.2 mL of 20 % chilled TCA. An equal volume of 0.2 M $NaIO_4$ was added to the deproteinized supernatant followed by a second incubation at 37ºC for 1h for the oxidation of the MIPS reaction product *myo*-insositol-1-phosphate, with concomitant release of inorganic phosphate. The excess periodate was destroyed by 1M Na_2SO_3. Simultaneously, appropriate non-periodate controls, without the $NaIO_4$ and Na_2SO_3 treatments, were also run. The activity of the enzyme was determined by estimating the product-specific release of inorganic phosphate from *myo*-insositol-1-phosphate formed in the MIPS reaction. The amount of inorganic phosphate released from the MIPS reaction product upon periodate oxidation was estimated by

the method of Chen et al. (1956) and protein was determined according to the method of Bradford (1976) with BSA as a standard. The protein content in fractions obtained from column chromatography was determined by measuring absorbance at 280 nm.

Protein electrophoresis: Polyacrylamide gel electrophoresis of the BioGel fraction was performed under non-denaturing conditions using 0.75 mm gels following the method of Bollag et al. (1996). The protein sample (30 µg/lane) was loaded onto a gel system containing 8 % separating gel and 4 % stacking gel and electrophoresed in a Biotech regular slab gel apparatus at 4ºC at 100 V. For MIPS activity in the gels, one of the replicate gels was sliced into 5 mm fragments and each fragment extracted with 250 µL of 50 mM tris-acetate buffer (pH 7.5) at 0ºC for 30 min. The extracts were then assayed for MIPS activity.

M_r determination: The apparent molecular weight of the native MIPS obtained from the reproductive pinnules of *D. glaucum* was determined by gel filtration through a Sephadex G-200 column. The molecular weight of the enzyme was determined from the relative elution volume of the active fractions by comparison with the standard curve prepared using as marker proteins, catalase (221.6 kDa), bovine serum albumin dimer (133.3 kDa), phosphorylase-b (97.4 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa) and lysozyme (14.3 kDa).

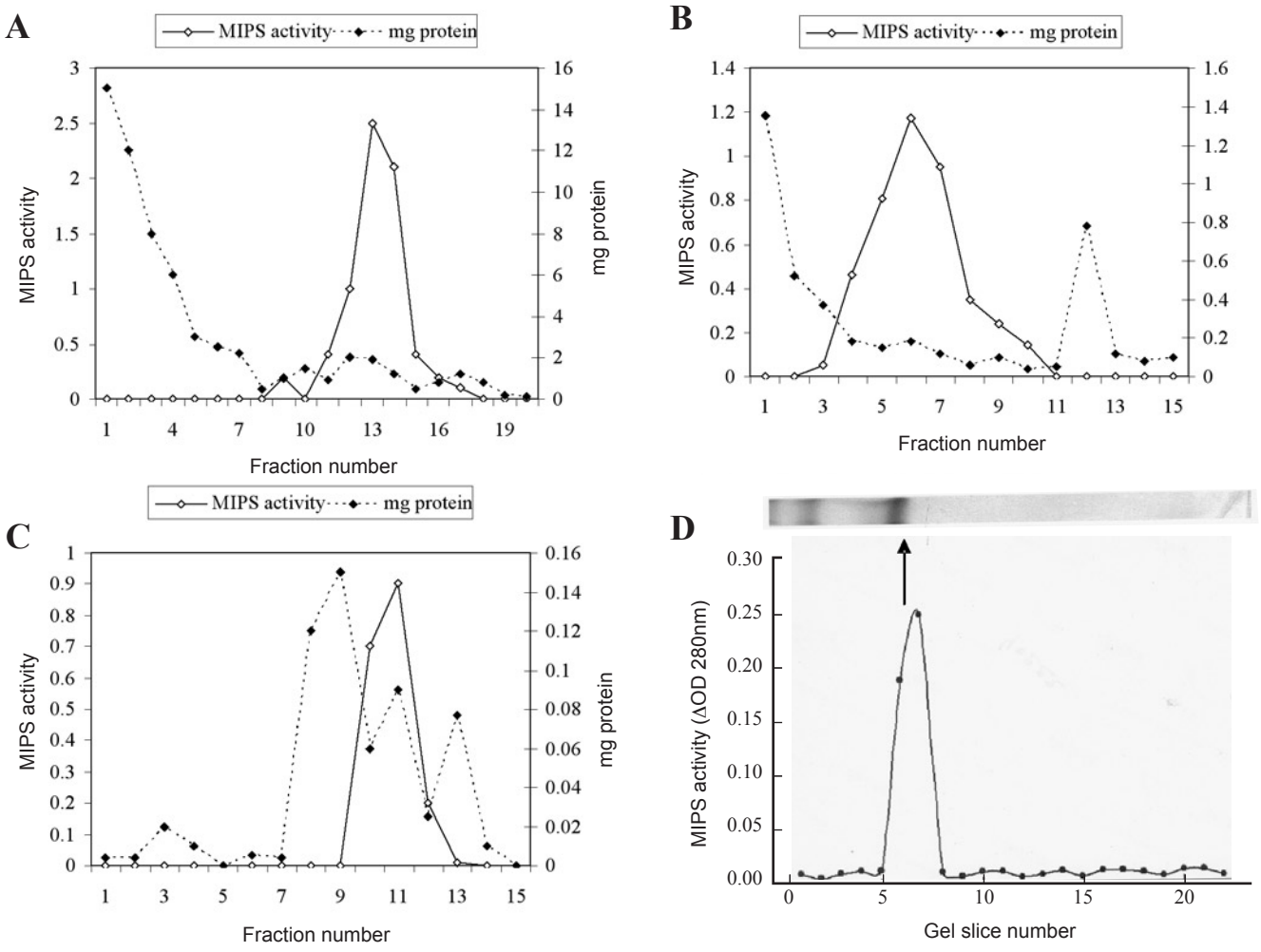

Figure 1. Elution profile of *D. glaucum* MIPS from a (A) DEAE-cellulose column [MIPS activity expressed as µmol I-1-P produced (2 mL fraction)$^{-1}$ h^{-1}], (B) Hexylagarose column [MIPS activity expressed as µmol I-1-P produced (1.25 mL fraction)$^{-1}$ h^{-1}], (C) Biogel A-0.5m column [MIPS activity expressed as µmol I-1-P produced (0.75 mL fraction)$^{-1}$ h^{-1}] and (D) PAGE profile showing the MIPS activity of the corresponding band.

Free myo-inositol determination: Free *myo*-inositol was isolated by the method of Charalampous and Chen (1966). The extracted sample was passed through a mixed-bed column of Dowex-1-Cl (100-200 mesh) and Amberlite IR-120 (Na-form) and the free *myo*-inositol was ultimately isolated by one dimensional descending chromatography on Whatman no.1 paper. The content of free *myo*-inositol was estimated spectrophotometrically (Gaitonde and Griffiths, 1966) using a standard curve prepared from known concentrations of pure *myo*-inositol.

RESULTS AND DISCUSSION

Determination of free myo-inositol *from pteridophytes*: Appreciable quantities of free *myo*-inositol (the final product of *myo*-inositol biosynthesis) were detected in vegetative and reproductive parts of different pteridophytic species (table 1). It was revealed that the quantities of free *myo*-inositol in almost all plant parts were moderately high. Free *myo*-inositol content was detected in relatively large quantities in the reproductive pinnules of *Lycopodium cernuum* and *Diplopterygium glaucum*. Different inositol derivatives are known to be essential for all life forms (Majumder et al., 2003). Hence, the detection of free *myo*-inositol in these pteridophytes is not surprising.

Purification of L-myo-inositol-1-phosphate synthase: The enzyme, MIPS was isolated and purified from the reproductive pinnules of *D. glaucum*. A summary of the purification of MIPS is given in table 2. Chromatographic profiles of proteins resolved from the $(NH_4)_2SO_4$ fraction of the crude homogenate of *D. glaucum* reproductive pinnules are shown in figure 1(A-C). The BioGel A-0.5m chromatography (figure 1C) revealed that the MIPS from *D. glaucum* was retained and eluted as a single sharp peak with the extraction buffer. In the present study, an overall purification of about 81-fold was achieved for this enzyme with about 13.5 % recovery based on total activity.

Table 1. Distribution of free *myo*-inositol in vegetative and reproductive structures of different pteridophytic species (Values are mean ± SE, n=3), FW = Fresh Weight.

Family	Plant species	Plant part	Free *myo*-inositol content (mg/g FW)
Gleicheniaceae	*Dicranopteris linearis* Bedd.	Vegetative Sori	0.70 ± 0.19 1.94 ± 0.14
Gleicheniaceae	*Diplopterygium glaucum* (Thunb.) Nakai	Vegetative Sori	2.50 ± 0.36 3.70 ± 0.61
Dryopteridaceae	*Diplazium dilatatum* Bl.	Vegetative Sori	1.20 ± 0.38 1.10 ± 0.11
Equisetaceae	*Equisetum debile* Roxb.	Vegetative Strobili	0.92 ± 0.10 0.60 ± 0.03
Lycopodiaceae	*Lycopodium cernuum* L.	Vegetative Strobili	2.88 ± 0.19 3.60 ± 0.20
Polypodiaceae	*Polypodium quercifolium* L.	Vegetative Sori	3.68 ± 0.31 0.80 ± 0.17
Hypolepidaceae	*Pteridium aquilinum* (L.) Kuhn	Vegetative Sori	0.82 ± 0.05 1.22 ± 0.12
Selaginellaceae	*Selaginella megaphylla* Bak.	Vegetative Strobili	3.52 ± 0.17 2.48 ± 0.12

Table 2. Summary of partial purification of L-*myo*-inositol-1-phosphate synthase from *D. glaucum* (Values are mean ± SE, n=3).

Purification step	Protein content (mg/mL)	Specific activity (µ mol I-1-P produced. mg^{-1} protein. h^{-1})	Total activity (µ mol I-1-P produced. mg^{-1} protein. h^{-1})	Recovery (%)	Purification (fold)
Homogenate	0.47 ± 0.05	0.12± 0.02	13.43 ± 5.55	100.00 ± 15.29	1.0 ± 0.15
DEAE-cellulose fraction	0.49± 0.05	1.21 ± 0.15	7.07 ± 1.30	52.66 ± 6.19	10.01 ± 1.61
Hexylagarose fraction	0.12 ± 0.01	5.02 ± 0.53	5.28 ± 0.50	39.34 ± 2.44	41.40 ± 1.93
BioGel fraction	0.04 ± 0.01	9.98 ± 2.15	1.81 ± 0.10	13.47 ± 1.08	80.99 ± 4.19

Requirements for G-6-P and NAD^+ for D. glaucum MIPS activity: The *D. glaucum* MIPS has been found to utilize G-6-P as the exclusive substrate since other hexose and pentose phosphates could not replace it. However, this enzyme could partially utilize D-galactose-6-phosphate (9.38 %) and D-mannose-6-phosphate (1.42 %). Omission of NAD^+ resulted in a loss of enzyme activity of about 67 %. In a similar study it was found that when NAD^+ was not added to the rice enzyme, its activity was reduced to 55 % of the maximum (Funkhouser and Loewus, 1975).

Enzyme stability: Stability of *D. glaucum* MIPS varied with the different stages of purification. While the low speed supernatant remained active for 20-22 days with insignificant loss of activity when stored at –20ºC, the BioGel-A 0.5m purified fraction maintained only about 80% of its activity up to 10-12 days when stored at an identical temperature. Addition of the enzyme stabilizers, 2-mercaptoethanol (ME) or dithiothreitol (DTT) increased the shelf-life of the enzyme by 7-10 days.

Enzyme concentration and the time linearity: The *D. glaucum* MIPS exhibited enzyme activity proportional to protein concentration up to 280 µg of protein under optimal assay conditions. The rate of enzyme reaction proceeded linearly up to 90 min with G-6-P as the substrate. An earlier study with *Acer pseudoplatanus* showed time linearity up to 150 min with a G-6-P concentration under 4 mM (Loewus and Loewus, 1971)

Effect of temperature and pH: The enzyme was highly active in the temperature range of 20-40ºC with 30ºC as the temperature maximum. Temperature maxima of 35ºC were found for the cytosolic enzymes from both higher and primitive plants (RayChoudhury et al., 1997). The *D. glaucum* MIPS exhibited optimum activity at a pH range of 7.0–7.5. MIPS from other plant sources exhibited similar pH optima (Gumber et al., 1984; RayChoudhury et al., 1997)

Effect of ammonium concentration: The *D. glaucum* MIPS activity was stimulated by NH_4^+ in a concentration-dependent manner up to 12 mM. The stimulation was to the order of 1.3-fold (approx.). Similarly, in yeast, maximum activity was not reached until the NH_4^+ concentration was raised to 10 mM (Chen and Charalampous, 1965). With NH_4Cl treatment, the enzyme activity was found to increase two-fold in *Euglena gracilis* (Dasgupta et al., 1984) and four-fold in *Entamoeba histolytica* (Lohia et al., 1999).

Effect of organic modifiers: Of the several organic compounds tried as effectors for the *D. glaucum* MIPS, three substrate isomers, Glucose-1-phosphate, Fructose-6-phosphate and Galactose-6-phosphate exhibited between 6 % and 14 % enzyme inhibition. On the other hand, the sulfhydryl binding

Table 3. Effect of some substrate isomers and pCMB on *D. glaucum* L-*myo*-inositol-1-phosphate synthase activity (Values are mean ± SE, n=3).

Compound	Concentration (mM)	Specific activity (µ mol I-1-P produced.mg^{-1} protein.h^{-1})	Percent activity
Control	0.0	9.166 ± 1.069	100.00 ± 11.66
D-glucose-1-phosphate	1.0	9.219 ± 0.394	100.56 ± 4.27
	2.5	8.711 ± 0.686	95.02 ± 7.88
	5.0	7.943 ± 0.652	86.64 ± 8.21
D-fructose-6-phosphate	1.0	9.037 ± 1.363	98.58 ± 15.08
	2.5	7.913 ± 0.375	86.33 ± 4.74
	5.0	7.863 ± 0.383	85.77 ± 4.87
D-galactose-6-phosphate	1.0	9.216 ± 1.356	100.53 ± 14.71
	2.5	9.028 ± 0.343	98.49 ± 3.80
	5.0	8.631 ± 0.805	94.16 ± 9.33
pCMB	1.0	6.852 ± 0.330	74.75 ± 4.82
	2.5	3.119 ± 0.079	34.03 ± 2.54
	5.0	2.524 ± 0.112	27.53 ± 4.83

agent, para-chloromercurobenzoate (pCMB) recorded 73 % inhibition for the enzyme at 5 mM concentration (table 3). The pine pollen MIPS was also inhibited to about 80 % by 0.1 mM pCMB (Gumber et al., 1984), however, it did not affect the enzyme activity of *Streptomyces griceus* (Sipos and Szabo, 1989).

Determination of K_m values: The K_m values of the substrate and the co-factor was determined by means of Lineweaver-Burk plots. The K_m value for G-6-P was found to be 0.83 mM and that of the co-factor NAD^+ was calculated as 0.44 mM. The K_m for G-6-P in case of *D. glaucum* MIPS is comparable to that of the yeast enzyme which has a value of 1.18 mM. However, the K_m for NAD^+ of the *D. glaucum* enzyme is quite different from that of the yeast enzyme which has a value of 8 mM (Donahue and Henry, 1981b).

Effect of monovalent and divalent cations: The effect of different metal ions was tested using chloride salts of metals. Of the monovalent cations tested K^+ had little effect, Na^+ played a minor inhibitory role and Li^+ was strongly inhibitory. In the case of divalent cations it was found that Ca^{2+} and Mg^{2+} exhibited no effect, Ba^{2+} slightly inhibited, Cd^{2+} moderately inhibited, while Zn^{2+} and Hg^{2+} strongly inhibited the enzyme activity (table 4).

Effect of sugar alcohols: The MIPS activity from *D. glaucum* was found to be little influenced by the presence of sugar alcohols like galactitol, mannitol and *myo*-inositol, the end product of the synthase and phosphatase reactions. However, synthase preparations from *Acer pseudoplatanus* cell culture (Loewus and Loewus, 1973) and *Arabidopsis thaliana* (Johnson and Sussex, 1995) are inhibited by the presence of inositol.

Effect of EDTA: In the concentration range of 0-100 mM, EDTA had a very significant effect on *D. glaucum* MIPS activity causing inhibition in a concentration-dependent manner. Earlier studies with other plants have shown that the production of MIPS is repressed by the addition of EDTA (Loewus and Loewus, 1983).

PAGE profile and corresponding enzyme activity: The MIPS activity was determined from 5 mm gel slices after the BioGel-A 0.5m fraction of *D. glaucum* was electrophoresed under non-denaturing conditions. Only a single major band of protein was found and this coincided with the position of the enzyme activity (figure 1D).

Molecular weight: The apparent molecular weight (M_r) of the native *D. glaucum* MIPS was determined by gel-filtration on a Sephadex G-200 column. The value of molecular mass was about 171 kDa. The molecular weight of *D. glaucum* MIPS was found to be comparable to the enzyme from other sources. The comparable M_r of MIPS from other reported sources are 155 from *Pinus ponderosa* pollen (Gumber et al., 1984), 179

Table 4. Effect of monovalent and divalent cations on L-*myo*-inositol-1-phosphate synthase activity in *D. glaucum*. Values are mean ± SE (n=3), ± SE is shown in the parenthesis.

Concentration of cations (mM)	Specific activity [µ mol I-1-P produced $(mg)^{-1}$ protein h^{-1}]								
	K^+	Na^+	Li^+	Ca^{2+}	Mg^{2+}	Ba^{2+}	Cd^{2+}	Zn^{2+}	Hg^{2+}
0	7.750 (1.12)	7.750 (1.12)	7.750 (1.12)	7.750 (1.12)	7.750 (1.12)	7.750 (1.12)	7.750 (1.12)	7.750 (1.12)	7.750 (1.12)
2	7.825 (0.69)	7.537 (1.22)	6.341 (1.37)	7.740 (0.50)	7.683 (0.08)	7.530 (0.66)	7.410 (0.21)	6.430 (0.83)	6.228 (0.61)
4	7.908 (0.58)	7.280 (1.06)	6.050 (0.52)	7.810 (0.76)	7.750 (0.46)	7.420 (0.32)	7.105 (0.24)	6.215 (0.57)	4.479 (0.79)
6	8.119 (1.62)	7.034 (1.34)	5.325 (1.01)	7.800 (1.01)	7.650 (0.57)	7.238 (0.75)	6.930 (0.15)	5.624 (0.23)	2.435 (0.18)
8	8.375 (1.40)	(6.250 (1.12)	4.296 (0.01)	7.618 (0.18)	7.684 (0.26)	6.966 (0.24)	6.439 (0.54)	4.845 (0.42)	1.013 (0.19)
10	8.108 (0.19)	6.000 (0.25)	3.328 (0.24)	7.716 (0.18)	7.817 (0.33)	6.750 (0.75)	5.610 (0.45)	4.190 (0.29)	0.500 (0.08)

from *Euglena gracilis* (RayChoudhury et al., 1997) and 180 from *Entamoeba histolytica* (Lohia et al., 1999).

DISCUSSION

Our objective was to determine the occurrence of *myo*-inositol in the pteridophyte group and partially purify the enzyme MIPS. It was found that appreciable quantities of free *myo*-inositol were present in all the species and plant parts examined. The accumulation may reflect the status of *myo*-inositol biosynthesis of each species. The apparent molecular weight of the *D. glaucum* MIPS native enzyme was determined as 170,828 Da which is consistent with the fact that in plants the molecular weight of the native cytosolic MIPS varies between 179 to 200 kDa (RayChoudhury et al., 1997). The enzyme from *D. glaucum* was highly specific for G-6-P. However, some enzyme activity was recorded when galactose-6-phosphate and mannose-6-phosphate were used as substrates. A NAD^+-dependent oxido-reductase, identical with MIPS, isomerizes galactose-6-phosphate to *muco*-inositol-1-phosphate (Adhikari and Majumder, 1988). In *D. glaucum,* the biosynthesis of *muco*-inositol through a metabolic by-pass cannot therefore be ruled out. Furthermore, the utilization of mannose-6-phosphate indicated the concomitant formation of another isomer of inositol. In this context the product identification of galactose-6-phosphate and mannose-6-phosphate utilization may open new avenues of research centered on the biosynthesis of inositol. Though the enzyme exhibits its optimal activity in presence of the co-enzyme NAD^+, it still could maintain about one third of the total activity when NAD^+ was not added exogenously. This is due to the presence of endogenous NAD^+ in the molecular architecture of this enzyme (Adhikari and Majumder, 1983, Dasgupta et al., 1984).

Of the monovalent cations, K^+ showed no significant effect while Li^+ exhibited exclusive inhibition of the *D. glaucum* enzyme in a concentration-dependent manner. The Li^+-induced decrease in MIPS activity may be attributed to the inhibition of inositol monophosphatase activity (Ju et al., 2004). Na^+ acted as a mild inhibitor of this enzyme as observed for the enzyme from other sources. Divalent cations also exhibited activities similar to those reported earlier from other sources (Wells et al., 1974; Loewus and Loewus, 1980). The strong enzyme inhibition due to heavy metals suggests that one or more free sulphydryl groups are present within the active site of the enzyme (Nelson and Cox, 2000). The *D.glaucum* MIPS was also inhibited by EDTA in a concentration-dependent manner which is a general feature of all plant enzymes (Dasgupta et al., 1984), in contrast to the same enzyme from animal systems (Maeda and Eisenberg Jr., 1980; Adhikari and Majumder, 1988).

Like all other eukaryotes, the *D. glaucum* MIPS requires NH_4^+ for optimal activity in contrast to the divalent cation-requiring MIPS of prokaryotes (Majumder et al., 2003). This indicates that the pteridophytic MIPS is a type-III aldolase. The present investigation presents the fundamental evidence for the classic occurrence of the enzyme MIPS and its ultimate product in pteridophytes. Further studies regarding the enzyme in this highly adaptive group of plants may pave the way for bioprospecting for useful genes.

Acknowledgements: The authors are grateful to the University Grants commission, Govt. of India, New Delhi for financing the present work with a minor research project (No. F. PSW-061/99-00 (ERO) to DRC.

REFERENCES

Adhikari J, Majumdar AL (1983) Differences in thermal stability of the fetal and adult brain *myo*-inositol-1-phosphate synthase. FEBS Lett. 163:46-49.

Adhikari J, Majumdar AL (1988) L-*myo*-Inositol-1-phosphate synthase from mammalian brain: partial purification and characterization of the fetal and adult enzyme. Ind. J. Biochem. Biophys. 25:408-412.

Adhikari J, Majumdar AL, Bhaduri TJ, Dasgupta S, Majumdar AL (1987) Chloroplast as a locale of L–myo-Inositol–1-phosphate synthase. Plant Physiol. 85:611-614.

Bachhawat N, Mande SC (1999) Identification of the INO 1 gene of *Mycobacterium tuberculosis* H37 Rv reveals a novel class of Inositol–1-phosphate synthase enzyme. J. Mol. Biol. 291:531-536.

Bachhawat N, Mande SC (2000) Complex evolution of the inositol-1-phosphate synthase gene among archea and eubacteria. Trends Genet. 16:111-113.

Barnett JEG, Brice RE, Corina DL (1970) A colorimetric determination of inositol monophosphates as an assay for D-Glucose–6-phosphate–1L-myo-Inositol-1- phosphate cyclase. Biochem. J. 119:183-186.

Benaroya RO, Zamski E, Tel-Or E (2004) L-myo-inositol-1-phosphate synthase in the aquatic fern *Azolla filiculoides*. Plant Physiol. Biochem. 42:97-102.

Bollag DM, Rozycki MD, Edelstein SJ (1996) Protein Methods. 2nd edn. Wiley-Liss, New York.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing principle of protein-dye binding. Anal. Biochem. 72:248-254.

Charalampous F, Chen IW (1966) Inositol-1-phosphate synthase and Inositol 1-phosphatase from yeast. Methods Enzymol. 9:698-704.

Chen IW, Charalampous FC (1965) Biochemical studies on inositol. VIII. Purification and properties of the enzyme system which converts glucose-6-phosphate to inositol. J. Biol. Chem. 240:3507-3512.

Chen RS, Toribara TY, Warner H (1956) Microdetermination of phosphorous. Anal. Biochem. 28:1756-1758.

Chen L, Zhou C, Yang H, Roberts MF (2000) Inositol-1-phosphate synthase from *Archaeoglobus fulgidus* is a class II aldolase. Biochemistry 39:12415-12423.

Chhetri DR, Choudhuri M, Mukherjee AK, Adhikari J (2005) L-myo-inositol-1-phosphate synthase: partial purification and characterization from *Gleichenia glauca*. Biol. Plant. 49:59-63.

Chiu TT, Rogers MS, Briton-Jones C, Haines C (2003) Effects of myo-inositol on the in-vitro maturation and subsequent development of mouse oocytes. Hum. Reprod. 18:408-816.

Chun JA, Jin UH, Lee JW, Yi YB, Hyung NI, Kang MH, Pyee JH, Suh MC, Kang CW, Seo HY, Lee SW, Chung CH (2003) Isolation and characterization of a myo-inositol 1-phosphate synthase cDNA from developing sesame (*Sesamum indicum* L.) seeds: functional and differential expression, and salt-induced transcription during germination. Planta 216:874-80.

Dasgupta S, Adhikari J, Majumdar AL (1984) Myo-inositol–1–phosphate synthase from lower plant groups: purification and properties of the enzyme from *Euglena gracilis*. Physiol. Plant. 61:412-416.

Donahue TF, Henry SA (1981a) Inositol mutants of *Saccharomyces cerevisiae*: Mapping the INO1 locus and characterizing alleles of INO1, INO2 and INO4 loci. Genetics 98:491-503.

Donahue TF, Henry SA (1981b) Myo-inositol-1-phosphate synthase: Characteristics of the enzyme and identification of its structural gene in yeast. J. Biol. Chem. 256:7077-7085.

Escamilla JE, Contreas M, Martinez A, Pina MZ (1982) L-myo-Inositol-1-phosphate synthase from *Neurospora crassa*: Purification to homogeneity and partial characterization. Arch. Biochem. Biophys. 218:275-285.

Funkhouser EA, Loewus FA (1975) Purification of myo-inositol-1-phosphate synthase from rice cell culture by affinity chromatography. Plant Physiol. 56:786-790.

Gaitonde MK, Griffiths M (1966) A spectrophotometric method for the determination of microquantities of free inositol in biological material. Anal. Biochem. 15:532.

Gumber SC., Loewus MW, Loewus FA (1984) Myo-inositol-1-phosphate synthase from pine pollen: Sulfhydryl involvement at the active site. Arch. Biochim. Biophys. 231:372–377.

Johnson MD, Sussex IM (1995) 1L–myo-Inositol 1-phosphate synthase from *Arabidopsis thaliana*. Plant Physiol. 107: 613-619.

Johnson MD, Wang X (1996) Differentially expressed forms of 1-L-myo-Inositol 1-phosphate synthase (EC. 5.5.1.4) in *Phaseolus vulgaris.* J. Biol. Chem. 271:17215-17218.

Ju S, Shaltiel G, Shamir A, Agam A, Greenberg ML (2004) Human 1-D-myo-inositol -3-phosphate synthase is functional in yeast. J. Biol. Chem. 279:21759-21765.

Lackey KH, Pope PM, Johnson MD (2003) Expression of 1L-myo-inositol-1-phosphate synthase in organelles. Plant Physiol. 132:2240-2247.

Loewus MW, Loewus FA (1971) The isolation and characaterisation of D-Glucose 6-phosphate cycloaldolase (NAD-dependent) from *Acer pseudoplatanus* L. cell cultures. Plant Physiol. 48:255-260.

Loewus MW, Loewus FA (1973) D-Glucose 6-phosphate cycloaldolase: inhibition studies and aldolase function. Plant Physiol. 51:263-266.

Loewus FA, Loewus MW, Maity IB, Rosenfield CL (1978) Aspects of myo-inositol metabolism and biosynthesis in higher plants. In: Wells WW, Eisenberg F Jr. (eds), Cyclitols and Phosphoinositides, pp. 249-267 Academic Press, New York.

Loewus FA, Loewus MW (1980) Myo-inositol: biosynthesis and metabolism. In: Preiss J (ed), The Biochemistry of Plants: Carbohydrate Structure and Function, Vol 3, pp.43-76. Academic Press, New York.

Loewus FA, Loewus MW (1983) Myo–Inositol: its biosynthesis and metabolism. Ann. Rev. Plant Physiol. 34:137-161.

Lohia A, Hait NC, Majumdar AL (1999) L-myo-inositol 1-phosphate synthase from *Entamoeba histolytica*. Mol. Biochem. Parasitol. 98:67-79.

Maeda T, Eisenberg F Jr. (1980) Purification, structure and catalytic properties of the 1 L-myo-inositol-1-phosphate synthase from rat testis. J. Biol. Chem. 255:8458–8464.

Majumder AL, Chatterjee A, Ghosh Dastidar K, Majee M (2003) Diversification and evolution of L–myo-inositol–1-phosphate synthase FEBS Lett. 553:3-10.

Nelson DL, Cox MM (2000) Lehninger Principles of Biochemistry. 3rd edn. Worth Publishers, New York.

Ogunyemi O, Pittner F, Hoffman-Ostenhof O (1978) Studies on the biosynthesis of cyclitols. XXXVI. Purification of myo-inositol-1-phosphate synthase of the duckweed *Lemna gibba* to homogeneity by affinity chromatography on NAD sepharose. Molecular and catalytic properties of the enzyme. Hoppe-Seyler's Z Physiol. Chem. 359:613–616.

Pittner F, Hoffman-Ostenhof O (1976) Studies on the biosynthesis of Cyclitols. XXXV. On the mechanism of action of myo-inositol 1-phosphate synthase from rat testicles. Hoppe-Scylers Z. Physiol. Chem. 357:1667-1671.

Pittner F, Hoffmann-Ostenhof O (1979) Preparation of homogeneous crystals of myo-inositol 1-phosphate synthase from rat testicles--further data on the chemical and catalytic properties of the enzyme (studies on the biosynthesis cyclitols, XXXIX). Mol. Cell. Biochem. 28:23-26.

RayChoudhury A, Hait NC, Dasgupta S, Bhaduri TJ, Deb R, Majumdar AL (1997) L-myo-inositol 1-phosphate synthase of plant sources. Plant Physiol. 115:727-736.

Sipos L, Szabo G (1989) Myo-inositol-1-phosphate-synthase in different S*treptomyces griseus* variants. FEMS Microbiol. Lett. 65:339-344.

Wells WW, Kuo CH, Naccarato WF (1974) Identification of 6-O-α-D-mannopyranosyl myo-inositol from *Saccharomyces cerevisiae.* Biochem. Biophys. Res. Commun. 61:644-650.