Occurrence of *myo*-inositol-1-phosphate phosphatase in pteridophytes: characteristics of the enzyme from the reproductive pinnules of *Dryopteris filix-mas* (L.) Schott

Ritwika Banerjee¹, Dhani R. Chhetri² and Jukta Adhikari¹*

¹Post-Graduate Department of Botany, Presidency College, 86/1 College Street, Kolkata – 700073, India. ²Post-Graduate Department of Botany, Darjeeling Govt. College, Darjeeling – 743101, India. *Corresponding author: adhikarijukta@gmail.com

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Evident *myo*-inositol-1-phosphate phosphatase (MIPP) activity has been detected both in the vegetative as well as in the spore-bearing organs of some selected pteridophytes having wide phylogenetic diversity. The basic characterization of this enzyme was carried out using the cosmopolitan fern *Dryopteris filix-mas*. The enzyme was partially purified from the cytosol fraction obtained from the reproductive pinnules of the plant to about 41-fold over the initial homogenate following low-speed centrifugation, streptomycin sulfate precipitation, 25-70% ammonium sulfate fractionation, CM Sephadex C-50 chromatography and finally gel-filtration on Ultrogel AcA 34. The apparent molecular weight of the native MIPP was estimated to be 94 kDa. The enzyme activity increased linearly with respect to protein concentration to about 150 µg and with respect to time up to 75 min. The temperature optimum was found at 40ºC. However, the enzyme showed good activity over the temperature range of 30-50ºC. This enzyme used D/L- *myo*-inositol-1-phosphate as its principal substrate (95-100%), however, about 16% activity was recorded when D- *myo*-inositol-3-phosphate substituted as substrate. Furthermore, weak (3%) activity of this MIPP was observed with 2-glycerophosphate as substrate. The apparent \( K_m \) for pteridophytic MIPP was 0.083 mM. The enzyme was functional in a narrow pH range of 7.5 to 8.5. The activity of this MIPP enzyme was remarkably inhibited by the presence of a monovalent cation, lithium, and even moderately so at a low concentration such as 1 mM. On the other hand, magnesium, a divalent cation, enhanced activity at least up to 10 mM. Calcium diminished MIPP activity at concentrations over 4 mM.

**Key words:** *Dryopteris filix-mas*, *myo*-inositol-1-phosphate phosphatase, pteridophytes, reproductive pinnules

Ocorrência da fosfatase do *mio*-inositol-1-fosfato em pteridófitas: características da enzima a partir de pínulas reprodutivas de *Dryopteris filix-mas* (L.) Schott: Tem-se detectado atividade da fosfatase do *mio*-inositol-1-fosfato (FMIF) tanto em órgãos vegetativos como em estruturas esporulantes de algumas pteridófitas com ampla diversidade filogenética. Neste estudo, procedeu-se à caracterização básica dessa enzima utilizando-se da pteridófita cosmopolita *Dryopteris filix-mas*. Após centrifugação em baixa velocidade, precipitação com sulfato de estreptomicina, fracionamento com sulfato de amônio (25-70%), cromatografia em CM Sephadex C-50 e, finalmente, filtração gélica em Ultrogel AcA 34, conseguiu-se uma purificação parcial da enzima (a partir da fração citosólica obtida de pínulas reprodutivas da planta) de cerca de 41 vezes em relação ao homogênato inicial. O peso molecular aparente da FMIF nativa foi estimado em 94 kDa. A atividade da enzima aumentou linearmente com relação ao conteúdo de proteína (cerca de 150 µg) e com relação ao tempo (até 75 min). A temperatura ótima foi de 40ºC. Entretanto, a enzima exibiu atividade razoável na faixa de temperatura entre 30 e 50ºC. O D/L-*mio*-inositol-1-fosfato foi o principal substrato (95-100%) da enzima, porém registrou-se uma atividade de cerca de 16% quando este composto foi substituído pelo D-*mio*-inositol-3-fosfato. Ademais, observou-se fraqueza de atividade (3%) da FMIF quando se utilizou o 2-glicerol-fosfato como substrato. O
INTRODUCTION

Inositols belong to a larger class of polyhydroxylated cycloalkanes, commonly known as cyclitols. Among the nine possible geometrical isomers of inositol, the myo-form is the most abundant in biological systems where it functions as an essential metabolite. It is present in a large number of organisms from microbial systems to higher plants and animals (IUPAC, 1976). Biosynthesis of myo-inositol is dependent on two enzymes. D-glucose-6-phosphate is irreversibly isomerised to L-myoinositol-1-phosphate by a NAD+-dependent oxidoreductase, L-myoinositol-1-phosphate synthase [EC 5.5.1.4; D-glucose-6-phosphate-1L-myoinositol-1-phosphate synthase (MIPS)] and the product of this enzymatic reaction (L-myoinositol-1-phosphate) generates free myoinositol on hydrolysis by a specific Mg²⁺-dependent phosphatase, myo-inositol-1-phosphate phosphatase [EC 3.1.3.25; D/L-myoinositol-1-phosphate phosphohydrolase, (MIPP)]. Inositol metabolism is indispensable for the development of plants, animals and some microorganisms being related with a large number of cellular and metabolic events. The crucial 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. Therefore, the variation in cellular concentration of myo-inositol is largely dependent upon the onset, offset and toggling between these two events by enzymatic regulations centered on MIPS and MIPP. Generation of free myo-inositol may be blocked if MIPP is not active. The MIPS reaction has been reported to occur in a large number of living systems namely archaea (Chen et al., 2000), bacteria (Bachhawat and Mande, 2000), protozoa (Lohia et al., 1999), lower plants (Donahue and Henry, 1981a,b; 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 et al., 1987; Johnson and Sussex, 1995; Johnson and Wang, 1996; Chun et al., 2003), and animals (Pittner and Hoffmann-Ostenhof, 1976; Maeda and Eisenberg Jr., 1980; Adhikari and Majumder, 1983, 1988; Chiu et al., 2003). In pteridophytes, Benaroya et al. (2004) and Chettri et al. (2005, 2006) have recently documented the occurrence and characterization of L-myoinositol-1-phosphate synthase. So far, work with regard to the participation of the subsequent enzyme in this metabolic sequence, myo-inositol-1-phosphate phosphatase (MIPP), has been carried out principally in animal systems (Eisenberg Jr, 1967; Attwood et al., 1988; Gee et al., 1988; Honchar et al., 1989; Leech et al., 1993; Pollack et al., 1994; Kwok and Lo, 1994; Fujimoto et al., 1996; Caselli et al., 1996), in archaea (Wang et al., 2006), in bacteria (Nigou and Besra, 2002), and in cyanobacteria (Patra et al., 2007). However, only a few reports are available of this enzyme from plant systems (Loewus and Loewus, 1983; Gumber et al., 1984). Therefore, although the inositol biosynthetic pathway is necessarily functional in the vascular cryptogams, the supporting evidence is based only on MIPS (Chhetri et al., 2005, 2006) without any information on MIPP. Hence the present investigation on MIPP is an attempt to bridge that gap and proceed with the study of this biosynthetic pathway through the evolutionary scale in pteridophytes centered on myo-inositol.

MATERIAL AND METHODS

Plant material: Experimental specimens of pteridophytes namely Lycopodium clavatum L., Selaginella megaphylla Bak., Equisetum elongatum Willd., Polypodium wallichii R. Br., Diploptrygium glaucum (Thunb.) Nakai, Pteridium aquilinum (L.) Kuhn. and Dicranopteris linearis Bedd. were collected freshly from
their natural habitats in and around the Darjeeling hills (ca. 2134 m a.s.l.) in the Eastern Himalayas. The specimens were kept frozen until use. *Dryopteris filix-mas* (L.) Schott, the principal specimen of this present investigation, was collected fresh from the localities in and around Thakurpukur, Kolkata, West Bengal, India. All experiments were carried out three times independently using different batches of partially purified enzyme.

**Chemicals:** D-myoinositol-1-phosphate (monosodium salt) and D-myoinositol-3-phosphate (sodium salt) were purchased from Ceylon Chemical, USA. D-glucose-6P (G-6-P, di-sodium salt), D-galactose-6P (di-sodium salt), D-fructose-6P (di-sodium salt), CM Sephadex C-50 (C50120), Ultrogel AcA 34 (U8878), dialysis bags, Coomassie Brilliant Blue G-250, Coomassie Brilliant Blue R-250 and BSA, were obtained from Sigma-Aldrich, USA. Protein molecular weight markers, namely Myosin rabbit muscle, Phosphorylase b, ovalbumin, and carbonic anhydrase were purchased from Genei, Bangalore, India. L-myoinositol-1-phosphate was prepared in this laboratory according to the method of Eisenberg Jr. (1967) on a small scale. 2-mercaptoethanol (ME), ammonium molybdate, acetic acid, ammonium sulphate, sodium hydroxide, potassium chloride, sodium carbonate, copper sulfate, lithium chloride, calcium chloride, magnesium chloride, Tris, trichloroacetic acid (TCA) and di-potassium hydrogen phosphate were purchased from E. Merck India Ltd., Mumbai, India. Ascorbic acid was from Sisco Research Laboratories, Mumbai, India. All other chemicals used were of analytical grade purchased from reputed Indian companies.

**Isolation and partial purification of MIPP:** Sample(s) (10 g each) of plant material were washed twice with cold glass-distilled water and homogenized in a chilled mortar and pestle in two volumes of 50 mM Tris-HCl buffer (pH 7.0) containing 0.2 mM of ME (hereafter referred to as standard buffer). Thereafter all the operations were carried out at 4°C. The crude homogenate was passed through three layers of muslin and the filtrate centrifuged at 1000 g for 5 min. The supernatant was again centrifuged at 11,400 g for 20 min in a Plasto Crafts Superspin-R centrifuge and the pellet discarded. The supernatant was dialyzed overnight against the standard buffer and the clear supernatant collected from the dialysis bag. This preparation was used for the basic screening experiments of all the specimens. Partial purification of MIPP was carried out using the mature reproductive pinnules of *Dryopteris filix-mas* going through the initial steps mentioned earlier followed by the successive purification steps. The 11,400 g supernatant obtained from the reproductive pinnules of *D. filix-mas* was subjected to streptomycin sulphate treatment at a final concentration of 1.5% (w/v) with constant stirring. The mixture was kept at 0°C for 30 min and then centrifuged at 11,400 g for 20 min. The supernatant (streptomycin sulphate-treated fraction) was collected and fractionated accordingly with 25-70% (NH₄)₂SO₄. The precipitated protein fraction was dissolved and dialyzed overnight using the standard buffer. The ammonium sulphate (25-70%) fraction was then loaded onto a column of CM Sephadex C-50 (1.0 x 13.0 cm), equilibrated with standard buffer. The effluent was collected. Then the column was washed with one bed volume of the same buffer and the proteins were eluted from the column with a linear gradient of 0.0 to 500 mM KCl prepared in standard buffer. Fractions of 2.0 mL were collected at a flow rate of 10 min per fraction (Figure 1). The active CM Sephadex C-50 fractions were pooled and chromatographed on an Ultrogel AcA 34 column (0.8 x 10 cm) and the proteins eluted with the standard buffer. Fractions of 1.0 mL were collected at a flow rate of 7 min per fraction (Figure 2). The active fractions of CM Sephadex C-50 from other batches of purification were pooled and concentrated before the characterization experiments. Active Ultrogel AcA 34 fractions were not chosen for this purpose, as the yield at this step was not appreciable.

**Assay of MIPP:** The myoinositol-1-phosphatase activity was assayed by the procedure of Eisenberg Jr (1967) with slight modifications. In a total volume of 1.0 mL, the incubation mixture contained 500 µL of 0.67 M Tris-HCl (pH 7.4) containing 50 mM MgCl₂, 400 µL of 154 mM KCl, 50 µL of 100 mM D-myoinositol-1-phosphate and an appropriate protein aliquot (50 µL / 100-200 µg). The reaction was started by addition of substrate immediately after the addition of the enzyme. The complete assay mixture was run along with an appropriate blank (without enzyme) and a zero time control in which 250 µL of 20% chilled TCA was added prior to the addition of the
enzyme. The enzymatic incubation was carried out for 60 min at 37°C. After 60 min the reaction was terminated by the addition of 250 µL of chilled TCA (20%). Two such sets (set I – experimental and set II – control, where myo-inositol-1-phosphate was replaced by an identical volume and concentration of D-fructose-6-phosphate) were run simultaneously each having one blank, one zero time control and one complete assay mixture. Inorganic phosphate was estimated by the method of Chen et al. (1956) with slight modifications. Protein was determined according to the method of Bradford (1976) with BSA as a standard. As 1 mol of myo-inositol-1-phosphate contains 1 mol of inorganic phosphate, the total mole number of inorganic phosphate released was equal to the total mole number of myo-inositol-1-phosphate hydrolyzed. The specific activity was defined either as nmol myo-inositol-1-phosphate hydrolyzed mg⁻¹ protein h⁻¹ or nmol P_i released mg⁻¹ protein h⁻¹.

**Protein electrophoresis:** Polyacrylamide gel electrophoresis of the Ultrogel AcA-34 fraction was performed under non-denaturing conditions using 1.0 mm gels following the method of Bollag et al. (1996). The protein sample (~20 µg per 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 4°C at 100 V. For MIPP activity in the gels, one of the replicate gels was sliced into 5 mm fragments and each fragment extracted with 300 µL of 50 mM Tris-acetate buffer (pH 7.5) at 0°C for 45 min. The extracts were then assayed for MIPP activity. SDS-PAGE was carried out principally according to the method of Laemmli (1970), subject to some necessary modifications, for proteins obtained from the important steps of purification.

**Molecular weight determination:** The apparent molecular weight of the native MIPP protein, obtained from the PAGE carried out with the active Ultrogel AcA-34 fraction, was determined with the GEL LOGIC 100 IMAGING SYSTEM (Kodak) using the “Molecular imaging software Kodak MI-V.4.0.5”. The system was calibrated previously with marker proteins of known molecular weights, namely myosin rabbit muscle (205,000 kDa), phosphorylase b (97,400 kDa), BSA (68,000 kDa), ovalbumin (43,000 kDa) and carbonic anhydrase (29,000 kDa).

**RESULTS**

Diverse representatives of pteridophytes were screened for the presence of MIPP. Table 1 depicts the results of such a survey. As evident, all the vascular cryptogams tested were found to have MIPP activity. The enzyme was functional in vegetative as well as in reproductive parts of the selected species. However, the reproductive parts showed much higher activity in comparison with the vegetative parts of the same species.
Table 1. Distribution of L-myoinositol-1-phosphate phosphatase (MIPP) in some members of pteridophytes. Specific activity was expressed as nmol L-myoinositol-1-phosphate hydrolyzed (mg protein)$^{-1}$ h$^{-1}$ in a dialyzed 11,400 g supernatant. Means ± SE. $n = 3$.

<table>
<thead>
<tr>
<th>Class</th>
<th>Family</th>
<th>Plant</th>
<th>Plant portion</th>
<th>MIPP specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lycopodiatae</td>
<td>Lycopodiaceae</td>
<td>Lycopodium clavatum</td>
<td>vegetative</td>
<td>34 ± 5.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>strobili</td>
<td>70 ± 11.70</td>
</tr>
<tr>
<td></td>
<td>Selaginellaceae</td>
<td>Selaginella megaphylla</td>
<td>vegetative</td>
<td>38 ± 8.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>strobili</td>
<td>74 ± 12.03</td>
</tr>
<tr>
<td>Equisetatae</td>
<td>Equisetaceae</td>
<td>Equisetum elongatum</td>
<td>vegetative</td>
<td>16 ± 1.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>strobili</td>
<td>24 ± 3.90</td>
</tr>
<tr>
<td>Filicatae</td>
<td>Polypodiaceae</td>
<td>Polypodium wallichi</td>
<td>vegetative</td>
<td>40 ± 6.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>sori</td>
<td>229 ± 23.65</td>
</tr>
<tr>
<td></td>
<td>Dryopteridaceae</td>
<td>Dryopteris filix-mas</td>
<td>vegetative</td>
<td>144 ± 24.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>sori</td>
<td>474 ± 32.91</td>
</tr>
<tr>
<td></td>
<td>Gleicheniaceae</td>
<td>Diplopterygium glaucum</td>
<td>vegetative</td>
<td>182 ± 9.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>sori</td>
<td>347 ± 18.77</td>
</tr>
<tr>
<td></td>
<td>Hypolepidaceae</td>
<td>Pteridium aquilinum</td>
<td>vegetative</td>
<td>140 ± 23.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>sori</td>
<td>408 ± 41.26</td>
</tr>
<tr>
<td></td>
<td>Gleicheniaceae</td>
<td>Dicranopteris linearis</td>
<td>vegetative</td>
<td>135 ± 16.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>sori</td>
<td>295 ± 11.52</td>
</tr>
</tbody>
</table>

Furthermore, it is also clear from the results of Table 1 that ferns do exhibit higher activities of this enzyme than the lower pteridophytic families on the basis of an evolutionary scale.

As *D. filix-mas* was readily available and exhibited excellent activity of MIPP in the mature reproductive pinnules, partial purification of MIPP was carried out from this cosmopolitan fern. Table 2 describes the outlines of purification of MIPP. This procedure enabled purification of the enzyme to 41-fold. The MIPP activity was determined from 5 mm gel slices after the Ultrogel AcA-34 fraction of *D. filix-mas* was electrophoresed under non-denaturing conditions. The major band of protein was found to coincide with the enzyme activity (Figure 3). Figure 4 presents the SDS-PAGE profile of the protein in the important and subsequent steps of purification. The apparent molecular weight of the native MIPP was estimated to be 94 kDa.

The partially purified *D. filix-mas* MIPP utilized D/L-myoinositol-1-phosphate as its substrate (95-100%), however, about 16% activity was recorded when D-myoinositol-3-phosphate was used as the substrate. Furthermore, weak (3%) activity of this MIPP was observed with 2-glycerophosphate as substrate (Table 3). The MIPP reaction was linear with time up to 75 min and protein concentration up to 150 µg. Fern MIPP exhibited appreciable activity within a temperature range of 20ºC to 50ºC having a maximum at 40ºC. The apparent $K_m$ value of MIPP for MIP was determined to be 0.083 mM by means of the Michaelis-Menten rate equation. The enzyme was found functional in a narrow pH range of 7.5 to 8.5 using 0.67 M Tris-HCl buffer (Figure 5). The activity of this MIPP enzyme was remarkably inhibited by the presence of a monovalent cation, lithium, and even moderately so at a low concentration such as 1 mM. On the other hand, magnesium, a divalent cation, enhanced activity at least up to 10 mM. Calcium diminished MIPP activity at concentrations over 4 mM (Figure 6).

**DISCUSSION**

Although MIPP activity has been previously reported for some flowering plant species (Loewus and Loewus 1983; Gumber et al., 1984), no investigation has so far been made to look for this activity among pteridophytes. Results presented here show the existence of MIPP in a variety of vascular cryptogams. Chhetri et al. (2005, 2006) have already established the occurrence of the prime enzyme of myo-inositol biosynthesis, MIPS, in pteridophytes. Therefore, the presence of MIPP adds
Table 2. Partial purification of cytosolic myo-inositol-1-phosphate phosphatase from the reproductive pinnules of Dryopteris filix-mas. The table represents a typical partial purification obtained from reproductive pinnules (35-37 g) of D. filix-mas. Protein concentration was expressed as mg mL⁻¹, specific activity as nmol Pi released (mg protein)⁻¹ h⁻¹, and total activity as nmol Pi released h⁻¹. Means ± SE. n = 3.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total volume (mL)</th>
<th>Protein concentration (mg)</th>
<th>Total protein (mg)</th>
<th>Specific activity (nmol Pi h⁻¹ mg⁻¹)</th>
<th>Total activity (nmol Pi h⁻¹)</th>
<th>Recovery (%)</th>
<th>Fold purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate fraction</td>
<td>72.0 ± 5.1</td>
<td>1.565 ± 0.3</td>
<td>112.7 ± 4.94</td>
<td>467 ± 24.6</td>
<td>52610 ± 134</td>
<td>100.00 ± 6.31</td>
<td>1.00 ± 0.05</td>
</tr>
<tr>
<td>11,400 g supernatant fraction</td>
<td>61.0 ± 4.8</td>
<td>1.56 ± 0.2</td>
<td>95.0 ± 4.5</td>
<td>471 ± 24.2</td>
<td>44767 ± 120</td>
<td>85.08 ± 5.29</td>
<td>1.00 ± 0.06</td>
</tr>
<tr>
<td>Streptomycin sulphate-treated</td>
<td>60.0 ± 4.2</td>
<td>1.54 ± 0.2</td>
<td>92.1 ± 3.9</td>
<td>472 ± 30.1</td>
<td>43485 ± 127</td>
<td>82.65 ± 6.80</td>
<td>1.01 ± 0.04</td>
</tr>
<tr>
<td>25-70 % (NH₄)₂SO₄ fraction</td>
<td>12.4 ± 0.9</td>
<td>6.01 ± 0.1</td>
<td>74.6 ± 0.7</td>
<td>510 ± 19.6</td>
<td>38044 ± 325</td>
<td>72.31 ± 3.16</td>
<td>1.09 ± 0.03</td>
</tr>
<tr>
<td>CM Sephadex C-50 fraction</td>
<td>8.0 ± 0.1</td>
<td>0.580 ± 0.030</td>
<td>4.64 ± 0.36</td>
<td>4814 ± 310</td>
<td>22337 ± 538</td>
<td>42.45 ± 1.45</td>
<td>10.3 ± 1.0</td>
</tr>
<tr>
<td>Ultrogel AcA34 fraction</td>
<td>3.0 ± 0.1</td>
<td>0.104 ± 0.040</td>
<td>0.312 ± 0.021</td>
<td>19426 ± 869</td>
<td>6061 ± 175</td>
<td>11.51 ± 2.64</td>
<td>41.6 ± 4.2</td>
</tr>
</tbody>
</table>

Figure 3. Native PAGE profile showing the myo-inositol-1-phosphate phosphatase (MIPP) activity of the corresponding band.

Activity of MIPP in native PAGE

strength to the ubiquitous occurrence of myo-inositol biosynthesis in pteridophytes. The partial purification of MIPP from D. filix-mas and its preliminary characterization is another step forward towards an understanding of the role of this enzyme in non-flowering vascular plants. Fern MIPP shares some common characteristics, such as substrate specificity, pH reliance and influence by monovalent cations like lithium, with other reported sources including microbes and mammals (Eisenberg Jr. 1967, Attwood et al., 1988; Gee et al., 1988; Honchar et al., 1989; Leech et al., 1993; Pollack et al., 1994; Caselli et al., 1996, Fujimoto et al., 1996, Nigou and Besra, 2002; Wang et al., 2006; Patra et al., 2007). However, its kinetic parameter, thermo-tolerance and calcium-dependent inhibition at lower concentrations, differ substantially in pteridophytic species.

Our concern in the present work has been three-fold. Firstly, MIPP has been reported in a diverse group of living organisms, but not in any pteridophyte. However, MIPS of pteridophytic origin is clearly shown here. Thus, this investigation has filled an obligatory gap in our complete understanding of inositol biosynthesis in pteridophytes with a wide phylogenetic connotation. Secondly, an excellent metabolic and structural synchronization can be established from this present study based on myo-inositol biosynthesis. We have observed previously (Chhetri et al., 2006) that MIPS activity is highest during the early stage of development
Table 3. Substrate specificity of cytosolic myo-inositol-1-phosphate phosphatase from Dryopteris filix-mas. Specific activity was expressed as nmol P\textsubscript{i} released (mg protein\textsuperscript{-1} h\textsuperscript{-1}). Concentrations for all substrates were 0.2 mM. Means ± SE. \(n = 3\).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-myo-Inositol-1-phosphate</td>
<td>4538 ± 352</td>
</tr>
<tr>
<td>L-myo-Inositol-1-phosphate</td>
<td>4604 ± 382</td>
</tr>
<tr>
<td>D-myo-Inositol-3-phosphate</td>
<td>754 ± 42</td>
</tr>
<tr>
<td>D-Glucose-6-phosphate</td>
<td>0.00</td>
</tr>
<tr>
<td>D-Galactose-6-phosphate</td>
<td>0.0</td>
</tr>
<tr>
<td>2-Glycerophosphate</td>
<td>307 ± 23</td>
</tr>
</tbody>
</table>

of the strobilus or sorus in a pteridophyte. The content of free myo-inositol increases during late stages of development of the strobilus or sorus. Activity of MIPP is also predominant in the mature spore-bearing organs of all the pteridophytes tested. Hence, we can suggest that MIPS triggers the synthesis of L-myo-inositol-1-phosphate during the juvenile stage of either stobilus or sorus development in a pteridophyte. This alcohol, myo-inositol, on hydrolysis by MIPP at the mature or later stages of strobilus or sorus development to fulfill the requirement of this essential metabolite for the developing spores. Thirdly, the search for MIPP protein can be eloquently achieved either by looking for immunologically cross-reactive material in different systems or more precisely by analysis of nucleotide sequences with the aid of a MIPP gene probe. For both of these techniques a purified enzyme is a pre-requisite. The present work is, therefore, a step forwards towards attaining this goal.

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OCCURRENCE OF MYO-INOSITOL-1-PHOSPHATE PHOSPHATASE IN PTERIDOPHYTES
