ABSTRACT

The fundamental screening of L-*myo*-inositol-1-phosphate synthase (EC 5.5.1.4) activity from the representative bryophyte species of Darjeeling hills was undertaken, which showed that the enzyme is distributed in all species of this cryptogrammic group studied. Similarly, the end product of the enzymatic reaction i.e., free *myo*-inositol was also found across the bryophytes of the different families under the study conditions. The reproductive part bearing plant bodies were richer sources of this enzyme as well as free *myo*-inositol in the selected samples, *Asterella khasiana* and *Sphagnum junghuhnianum*. L-*myo*-inositol-1-phosphate synthase (MIPS) was partially purified from the reproductive part bearing thallus of *Asterella khasiana* and *Sphagnum junghuhnianum.* The purification procedure involved homogenization, low speed centrifugation, streptomycin sulphate precipitation, ammonium sulphate fractionation, chromatography on DEAE cellulose, Hexylagarose and Bio Gel-A 0.5 m. The enzyme was purified to about 46.34 fold over the homogenate fraction with 36.35% recovery from the liverwort, *A. khasiana*. Similarly, from the moss, *Sphagnum junghuhnianum*, a purification of about 58.67 fold over its homogenate fraction with about 32.86% recovery based on total activity was achieved. Apparent molecular weight of bryophytic MIPS from *Asterella khasiana was* approximately 183kDa and that from *Sphagnum junghuhnianum* was determined as approximately 174 KDa.

D-glucose-6-phosphate was found to be the specific substrate for MIPS from the bryophytes. Among the other hexose phosphates, D-glucose-6-phosphate, D-glucose-1-phosphate, D-fructose-6-phosphate and D-fructose-1,6-bisphosphate could not act as substrates for this enzyme. However, the enzyme showed a little bit of activity in

presence of D-galactose-6-phosphate and mannose-6-phosphate. With the increase in substrate (D-glucose-6-phosphate) concentration from 0 to 6 mM and coenzyme (NAD^+) concentration from 0 to 0.8 mM the activity of *A. khasiana* MIPS increased linearly. The *A. khasiana* MIPS showed K_m values of 3.56 mMand 0.56 mM for D-glucose-6-phosphate and NAD^+ respectively while the V_{max} values were found to be 0.71 mM and 0.68 mM for D-glucose-6-phosphate and NAD^+ respectively. In comparison, the activity of *S. junghuhnianum* MIPS increased linearly with the increase of D-glucose-6-phosphate concentration from 0 to 6 mM and coenzyme (NAD^+) concentration from 0 to 0.6 mM. The K_m for D-glucose-6-phosphate and NAD^+ were 1.81mM and 0.25mM respectively while the V_{max} values were worked out to be 1.42 mM and 1.12 mM for D-glucose-6-phosphate and NAD^+ respectively.

Stability of the bryophytic MIPS varied with the enzyme preparation at different stages of purification. In *Asterella khasiana*, the low speed supernatant remained active for 7-8 days and the BioGel purified fractions for 3-4 days when stored at −20 ºC. Comparatively, the low speed supernatant and BioGel purified fractions from *Sphagnum junghuhnianum* maintained its activity for 10-12 days and 5-7 days respectively, when stored at identical temperature. The deduction of NH_4Cl and ME reduced MIPS activity to 31.80% and 34.51% respectively in of *A. khasiana* and the same caused 40.01% and 33.35% loss of activity respectively in *S. junghuhnianum.* No enzyme activity was observed in absence of glucose-6-phosphate (substrate) and in case of heat-killed enzyme in either of the samples *Asterella khasiana* and *Sphagnum junghuhnianum*. However, when NAD^+ was deducted from the reaction mixture, the enzyme from *A. khasiana* exhibited approximately 59.17% loss of activity and that from *S. junghuhnianum* exhibited about 68.41% loss of activity.

The activity of bryophytic MIPS was directly proportional to the time of incubation upto 90 minutes in both cases and with respect to protein concentration upto 250µg in *A. khasiana* and 300 µg in *S. junghuhnianum*. The bryophytic MIPS operated between pH ranges of 7.0 to 7.5. However, the maximum activity was found at pH 7.0. Though MIPS from both the sources showed temperature maxima at 30^{o}C, it showed significant activity at temperatures as low as 20^{o}C in *A. khasiana* and 10^{o}C in case of *S. junghuhnianum.*

NH_4Cl was a strong stimulator of the enzyme and increased the rate of reaction in a concentration guided manner by 7.5 and 9.3 fold in *A. khasiana* and *S. junghuhnianum* respectively. Similarly, $MgCl_2$ acted as a mild stimulator, increasing the enzyme activity up to 1.6 fold and 1.2 fold in *Asterella khasiana* and *S. junghuhnianum* respectively. EDTA acted as a mild inhibitor in both the cases. Among the monovalent cations, K^+ was mildly stimulatory, Na^+ mildly inhibitory, while Li^+ was strongly inhibitory to the enzyme. Among the divalent cations studied, Ca^{2+} was mildly stimulatory, while Mn^{2+}, Cu^{2+} and Cd^{2+} had varying degree of inhibitory effect from mild to medium. Zn^{2+} was a strong inhibitor of the enzyme while Hg^{2+} showed extreme inhibitory property towards the bryophytic MIPS.