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BIOSYNTHESIS OF *MYO*-INOSITOL IN CHLOROPLASTS OF SALINITY-STRESSED MARINE MACRO ALGA *ULVA LACTUCA*

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Abstract

Basu S., Basak A., Mahanty D.S., Bhattacharjee S., Adhikari J., 2019: Biosynthesis of *myo*-inositol in chloroplasts of salinity-stressed marine macroalga *Ulva lactuca.* – Botanica, 25(1): 32–40.

The present communication reports enhanced *myo*-inositol biosynthesis under natural conditions in *Ulva lactuca* Linn. based on the study conducted on its two prime enzymes [L-*myo*-inositol-1-phosphate synthase (MIPS) and *myo*-inositol-1-phosphate phosphatase (MIPP)] involved in *myo*-inositol biosynthesis. The two key enzymes obtained from chloroplastidial sources were partially purified to about 49- and 58-fold, respectively, over the homogenate following low speed centrifugation, high speed centrifugation, 0–80% ammonium sulphate precipitation and successive chromatography using ion exchange, gel filtration and molecular sieve packed columns. MIPS preparations specifically utilized D-glucose-6-phosphate and NAD as its exclusive substrate and coenzyme, while MIPP preparations used D/L-*myo*-inositol -1- phosphate as its principal substrate. Using non-linear regression kinetics method, the Km values of MIPS for G-6-P and NAD were calculated to be 2.6340 mM and 0.1271 mM, while the Km value of MIPP for D-MIP was recorded to be 0.02128 mM. Both enzymes were remarkably active within a temperature range of 20–40°C, and the optimum pH for both enzymes were found to be 7.5. Different cations and organic modifiers exhibited variable effects on the activity of both enzymes. The content of free *myo*-inositol was found to increase proportionately with the increase of surface salinity of the Chilika Lagoon, Odisha, India.

Keywords: Chloroplast, L-*myo*-inositol-1-phosphate synthase (MIPS), marine macroalga, *myo*-inositol-1-phosphate phosphatase (MIPP), salinity tolerance, *Ulva lactuca*.

Abbreviations: G-6-P – D-Glucose-6-phosphate; MIP – *myo*-Inositol-1-phosphate; ME – 2-Mercaptoethanol; MIPP – *myo*-inositol-1-phosphate phosphatase, MIPS – L-*myo*-inositol-1-phosphate synthase, MI – *myo*-Inositol; PSU – Practical Salinity Unit.

INTRODUCTION

myo- Inositol is a 6-carbon cyclohexane hexitol, which participates in many different aspects of plant metabolism. It is required either in its free form or in different conjugated forms as a major component of reproductive units, precursors of storage phosphates in seeds, pollen cell wall polysaccharides (Murashige & Skoog, 1962; Maiti & Loewus, 1978; Chiu et al., 2003) and signal molecules (Sekhar & Hokin, 1986; Majumder & Biswas, 2006). In addition to these, it is also a precursor and substrate of many crucial metabolites in plants (Saxena et al., 2013). The *de-novo* biosynthesis of this important sugar alcohol is dependent on two enzymes, out of which the first one is a rate limiting NAD^+ dependent oxi-

doreductase (L-*myo*-Inositol-1-phosphate synthase; MIPS; EC 5.5.1.4) and the second one is a Mg^{+2} dependent phosphatase (D/L- *myo*-Inositol-1-phosphate phosphatase; MIPP; EC 3.1.3.25). L-*myo*-inositol-1-phosphate synthase (MIPS) carries out the irreversible conversion of D-Glucose- 6-phosphate (G-6-P) to L-*myo*-inositol-1-phosphate (I-1-P), which is subsequently dephosphorylated by inositol monophosphatase (MIPP) to free *myo*-inositol (MI) (Eisenberg, 1967).Together these two enzymes interact with each other in inositol biosynthesis metabolon. The MIPS and MIPP reactions have been reported from several pro- as well as eukaryotic sources covering a wide phylogenetic diversity (Eisenberg, 1967; Maeda & Eisenberg, 1980; Loewus & Loewus, 1983; Adhikari & Majumder, 1983; Gumber et al., 1984; Lohia et al., 1999; Chen et al., 2000; Majumder & Biswas 2006; Chhetri et al., 2006a, b; Patra et al., 2007; Banerjee et al., 2007; Basak et al., 2012). In this context, it is noteworthy to mention that although few studies on MIPS have been performed from various living systems inclusive of plants; however, studies concerning MIPP, specifically in plants, are surprisingly very limited. Although the inositol biosynthetic pathway has been found to be operative in vascular cryptogams and flowering plants (Loewus & Loewus, 1983; Gumber et al., 1984; Adhikari et al., 1987; Chhetri et al., 2006a, b; Banerjee et al., 2007; Basak et al., 2012), specific information regarding its functionality in non-vascular cryptogams is a neglected area of research. Taking these facts into consideration, attempts were made in the present investigation on purification and characterization of chloroplast localized MIPS and MIPP from a Chlorophycean marine macroalga *Ulva lactuca* Linn. (known commonly as sea lettuce), which has been reported to grow profusely in the Chilika Lagoon (Odisha, India) throughout all seasons under different salinity regimes (Rath & Adhikary, 2005). The plastidial or particulate forms of MIPS and MIPP are more significant than its cytosolic or soluble iso-forms in connection to salinity tolerance (Gill et al., 2014). To our knowledge, this is the first report that describes a protocol for comparative purification and characterization of chloroplstidial MIPS and MIPP from *U. lactuca*, salinity-tolerant macroalga grown under its natural habitat.

MATERIALS AND METHODS

Sample collection

Experimental macroalga *Ulva lactuca* was collected from the Kalijai Island, the Chilika Lagoon, Odisha, (geographic location: 19°50' N and 85°30' E), India. The samples were kept frozen under –20° C until use.

Isolation of chloroplasts

Isolation of chloroplasts from turgid thalli of *Ulva lactuca* (collected in winter season, when the lagoon water salinity remains high) were carried out after the method of Hachtel (1976) with minor modifications as suggested by Adhikari et al. (1987). Green tissues were collected fresh and washed several times with distilled water, allowed to drain off and placed in a plastic bag in refrigerator for few hours until these were turgid. Thereafter, all operations were carried out at 0°C to 4°C. Green tissues (50 g) were homogenized in a mortar and pestle with double volume of 20 mM Tris-acetate (pH 7.0) containing 0.35 M sucrose, 10 mM $MgCl_2$, 10 mM KCl, 1 mM 2-mercaptoethanol (ME) and 10 mM sodium ascorbate. Homogenization was carried out with equilibrated neutral sand (in 20 mM Tris-acetate, pH 7.0). The crude homogenate was centrifuged at 435 × g for 5 min in a Remi C-24 BL centrifuge. The pellet containing the unbroken cells, nuclei, other debris and sand was discarded. The supernatant obtained was spun at 2850 × g for 15 min in a Remi C-24 BL centrifuge. The resultant chloroplast pellet was washed at least three times with the homogenizing buffer.

Partial Purification of MIPS and MIPP

To purify the MIPS and MIPP from the experimental samples separately, all operations were carried out at 0–4°C. About 50 g of plant tissue-derived chloroplast pellet were thoroughly washed with sterile cold distilled water twice. The tissues were then homogenized in a mortar and pestle with two volumes of 50 mM Tris-acetate buffer (pH 7.5) [standard buffer] containing 0.2 mM ME with neutral sand. The homogenate was spun at 11 400 × g for 30 min in a refrigerated centrifuge (Remi C-24 BL) and the supernatant was saved. The resultant low-speed supernatant was then centrifuged at 104 000 × g in an ultracentrifuge (Hitachi GX Series, Model: CS 120G × 2 Micro Ultracen-

trifuge) for two hours. On completion of the centrifugation, the clear supernatant (High-speed supernatant) was collected from the centrifuge tubes and kept ready for the next step. The high-speed supernatant was fractionated with ammonium sulphate (0–80% saturation). The resultant pellet was dissolved in minimal volume of standard buffer and dialyzed overnight against the same (at least against 500-600 vols). Dialyzed fraction was separately chromatographed using an anion-exchange matrix DEAE-cellulose in a glass column for MIPS and cation exchange matrix CM-cellulose for MIPP. The respective effluent was collected and the column was washed with one bed volume of the standard buffer. The elution of adsorbed proteins was made by a linear gradient of 0 to 0.5 M KCl prepared in standard buffer. The MIPS active DEAE-cellulose fractions and MIPP active CM-cellulose fractions were pooled individually and gel filtration was made through Sephadex G-200 column independently. The MIPS active Sephadex G-200 fractions were pooled together and another molecular sieve chromatogram was made through BioGel A 0.5m column. The MIPP active Sephadex G-200 fractions were combined and subsequently the final chromatogram was made using Ultrogel AcA 34. The MIPS/MIPP active BioGel A 0.5 m/Ultrogel AcA 34 fractions were combined together as the MIPS/MIPP concerned and used as the enzyme preparation(s) for biochemical characterization of MIPS/MIPP, correspondingly.

Assay of MIPS and MIPP

Assay of L-*myo*-inositol-1-phosphate synthase was carried out by the procedure of Barnett et al. (1970) with slight modifications (Adhikari et al., 1987). The MIPP activity was assayed by the procedure of Eisenberg (1967) with minor modifications as suggested by Banerjee et al. (2007). The inorganic phosphate was estimated by the method of Chen et al. (1956).

Quantification of protein

Protein was determined according to the method of Bradford (1976) with BSA (Bovine Serum Albumin) as a standard.

Determination of *Mr*

The *Mr* (relative molecular mass) of the native MIPS and MIPP obtained from the respective samples was determined by gel-filtration through Sephadex G-200 using marker proteins namely catalase (221.6 kDa), bovine serum albumin dimer (133.352 kDa), phosphorylase-b (97.4 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa) and lysozyme (14.3 kDa). All standards were loaded in the column separately and fractions (1.0 ml) were collected at a flow rate of 1.0 ml/8 min. Each individual protein peak was located by spectrophotometric scanning at 280 nm in a Systronics UV-Vis Spectrophotometer (Model No: 118). A standard curve was prepared by plotting relative elution volume of proteins against their respective log molecular weights.

Determination of thermal sensitivity

Enzyme incubation was separately carried out at 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60^{o}C within multiple temperature controlled incubator.

Determination of pH sensitivity

Enzyme incubation was carried out separately using Tris-acetate buffer having pH 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0.

Analyses of Enzyme Kinetics

All kinetic parameters (for both MIPS & MIPP) were calculated by nonlinear regression kinetics method using the software Prism 7 (Graph Pad).

Determination of free *myo*-inositol

Free *myo*-inositol was isolated by the method of Charalampous & Chen (1966) and quantified after the method of Gaitonde & Griffiths (1966).

Determination of PSU

Seasonal variation in the salinity level (in terms of Practical salinity Units/PSU) of the Chilika Lagoon was measured using Systronics Water Analyzer (Model no: 571).

Statistical data analysis

Statistical data of the relevant experiments (in triplicate) were determined using Prism 7 software (Graph Pad).

RESULTS AND DISCUSSION

When screened for their activity under low and high salinity conditions it was realized that both cy-

tosolic as well as plastidial forms of MIPS and MIPP were appreciably active in case of the experimental model alga *U. lactuca* (Table 1). However, compared to the cytosolic iso-forms, a higher titre of both MIPS and MIPP were recorded in salinity-stressed chloroplasts (Table 1). Substantial increment in the content of free *myo*-inositol was noticed in winter season (under higher salinity niche) and this increment was correlated with the enhanced activity of both plastidial MIPS and MIPP as expected. This augmentation in the content of free *myo*-inositol in the chloroplasts was also accompanied with a proportional increase in surface salinity of the Chilika Lagoon (Table 1). Coastal lagoons such as the Chilika undergo a cyclical variation in salinity throughout the year and the level of salinity always plays a vital role in influencing the distribution of flora and fauna (Rath & Adhikary, 2005). It has been reported earlier that metabolic status of *myo*-inositol (MI) beyond biosynthesis produces several stereo-forms of inositols, which play a plethora of functional role in plants, including protection unequivocally against salt stress. Isomerization and methylation of MI leads to the formation of *O*-methyl inositols, which directly participate in stress-related responses of plants (Loewus & Murthy, 2000). Thus, the enhancement at the level of plastidial inositol pool in response to enhanced salinity level of the Chilika may be attributed to the osmo-protective role played by inositol and/or its derivatives in order to boost the adaptive and survival strategy of *U. lactuca.* However, it cannot be stated with certainty that the enhanced intracellular inositol pool is exclusively created as a result of *de novo* biosynthesis. The possible involvement of an alternative recycling/scavenging pathway cannot also be ruled out, which one is yet to be studied.

Although, the results obtained in the present investigation are from chloroplastidial preparations (for both MIPS and MIPP), it is noteworthy to mention that the basic characterization parameters are in good concurrence with its cytosolic counterparts studied from various sources. In the present investigation, an overall purification of MIPS was recorded to be around 49-fold with about 35% recovery, while in case of MIPP, the purification rate was found to be about 58-fold with nearly 49% yield (Table 2). The apparent molecular weights of the native plastidial MIPS and MIPP were recorded to be about 162 kDa

Table 1. Profile of free *myo*-inositol content in the experimental alga *Ulva lactuca* along with *myo*-inositol-1-phosphate phosphatase (MIPS) and L-*myo*-inositol-1-phosphate synthase (MIPP) activities in the cell free extracts

Collection site	Sub cellular locale	Collection season	Salinity of water, PSU	Free *myo*-inositol content, mg g^{-1} FW	Specific activity of MIPS, n mole I-1-P produced mg^{-1} protein h^{-1}	Specific activity of MIPP, n mol I-1-P hydrolized mg^{-1} protein h^{-1}
Kalijai Island (Barkul), Chilika Lagoon	Cytosol	Monsoon	8.56–8.90	1.14 ± 0.15	47.52 ± 12.04	23.52 ± 6.11
	Chloroplast			2.13 ± 0.24	88.12 ± 8.95	39.84 ± 12.22
	Cytosol	Winter	11.73–13.22	1.78 ± 0.43	34.76 ± 7.62	28.33 ± 7.40
	Chloroplast			3.98 ± 0.66	140.02 ± 15.33	52.71 ± 5.26

Table 2. An over-view of major biochemical characteristics of chloroplastidial *myo*-Inositol-1-phosphate synthase (MIPS) and L-*myo*-inositol-1-phosphate phosphatase (MIPP) in the experimental alga *Ulva lactuca*

MIPS								MIPP					
Final purification n fold in biogel A-0.5m fraction	Substrate specificity (G-6-P)	Coenzyme specificity (NAD)	Temperature optimum	pH optimum	Km for G-6-P (substrate), mM	Km for NAD, mM	Relative molecular mass, KDa	Final prification fold in Ultrogel AcA 34 fraction	Substrate specificity (D-MIP)	Temperature optimum	pH optimum	Km for MIP (substrate), mM	Relative molecular mass, KDa
49 fold	4938.65	4893.77	30 (20–40)	7.5	2.6340	0.1271	162	58 fold	2675.15	35 (20–40)	7.5	0.02128	142

and 142 kDa, respectively (Table 2). Perusal of available literature clearly reveals that the molecular weight of native plastidial MIPS has been found to vary between 155 kDa (*Diplopterygium glaucum*) (Chettri et al., 2006b) and 180 kDa (*Entamoeba histolytica*) (Lohia et al., 1999). The molecular weight of native cytosolic MIPP from a common fern *Dryopteris filix-mas* has been reported to be around 94 kDa (Banerjee et al., 2007), but no specific information is available regarding the molecular weight of its plastidial iso-form in that fern genus. In the present communication, however, an attempt was made to fulfil this lacunae existing in previous reports.

In characterization experiments of both MIPS and MIPP in the experimental alga *U. lactuca*, both enzymes recorded maximum activity (100%), when assayed in the presence of all the assay components. About 6% activity was lost in case of MIPS in the absence of Tris buffer. On the other hand, elimination of NAD^+, NH_4Cl and ME from the reaction mixture resulted in about 45, 9 and 2% drop of MIPS activity. Since the plastidial MIPS obtained from *U. lactuca* exhibited substantial activity in the absence of NAD^+, it can be anticipated that the plastidial MIPS from *U. lactuca* may contain 'built- in' NAD^+ in its molecular architecture. This is in complete agreement with the results obtained earlier in case of *Euglena gracilis* (Dasgupta et al., 1984) and *Diplopterygium glaucum* (Chettri et al., 2006a). MIPS from various sources have been reported to contain bound NAD^+ and the presence of such endogenous coenzyme is said to enhance the thermo-tolerance and stability of plastidial MIPS as suggested by Chettri et al. (2006b). The activity of plastidial MIPP, on the other hand, dropped by about 81% in the absence of buffer and 84% in the absence of KCl. Concerning the determination of substrate and coenzyme specificities for MIPS, G-6-P was found to be the exclusive substrate for the production of MIP and other hexose phosphates (viz, D-glucose-1, 6-bisphosphate, D-fructose-6-phosphate, D-fructose-1, 6-bisphosphate) under identical concentration (8.0 mM) replacing G-6-P were ineffective as substrate(s). In the experimental algal MIPS, NAD^+ was found to be the elite coenzyme for the production of MIP and other possible compounds, namely NADP, FAD or FADP could not replace the coenzyme dependence of MIPS. The algal MIPP, on the other hand, utilized either L-MIP or D-MIP as its exclusive substrate, almost with equal efficiency, and other hexose phosphates (viz, D-glucose-6-phosphate, D-fructose-6-phosphate and D-galactose-6-phosphate) under similar (1.0 mM) concentration could not replace the principal substrate D/L-MIP. However, a weak plastidial MIPP activity (15%) was recorded when D-*myo*-inositol- 3-phosphate replaced the principal substrate. This is in complete concurrence with the results obtained in case of *Dryopteris filix-mas* (Banerjee et al., 2007).

Both MIPS and MIPP obtained from *U. lactuca* were found to be remarkably active between the temperature ranges of 20–40°C. However, the temperature maxima for MIPS and MIPP were recorded to be about 30 and 35°C, respectively. The temperature optima for plastidial MIPS recorded in the present investigation is in agreement with most previously published reports. Few conflicting reports also exist, which have revealed lower temperature maxima for plastidial MIPS (Chettri et al., 2006b). A quick glance on the reports published earlier have clearly revealed a marked variation in the temperature maxima of MIPP obtained from different sources (Basak et al., 2012; Patra et al., 2007). In case of the experimental alga, both enzymes were found to be optimally active at pH 7.5. Plastidial MIPS obtained from different sources has been reported to exhibit narrow pH optima, while information regarding the pH reliance of plastidial MIPP from plant systems is meagre or none.

In kinetic analyses for MIPS, using G-6-P (as substrate), the reaction rate was noted to increase with respect to G-6-P concentration of 3 to 4 mM (when tried between a concentrations range from 0.0 to 10 mM at an interval of 1.0 mM). The Km value for G-6-P, as determined by the Michealis-Menten equation (using Prism 7 software), was 2.6340 mM; using NAD^+ (as coenzyme), the activity was found to increase up to 0.7 mM (when tried between a concentrations range from 0.0 to 1.0 mM at an interval of 0.1 mM), and the Km value for NAD^+ was detected to be 0.1271 mM (using Prism 7 software) (Fig. 1a, 1b). The kinetic analysis for MIPP was carried out using D-MIP (as substrate). The reaction rate was found to increase up to a substrate concentration of 0.1 to 0.2 mM (when tried between a concentrations range from 0.0 mM to 1.0 mM at an interval of 0.1 mM), and the Km value for D-MIP was recorded to be 0.02128 mM using Prism 7 software (Table 1; Fig. 1 c).

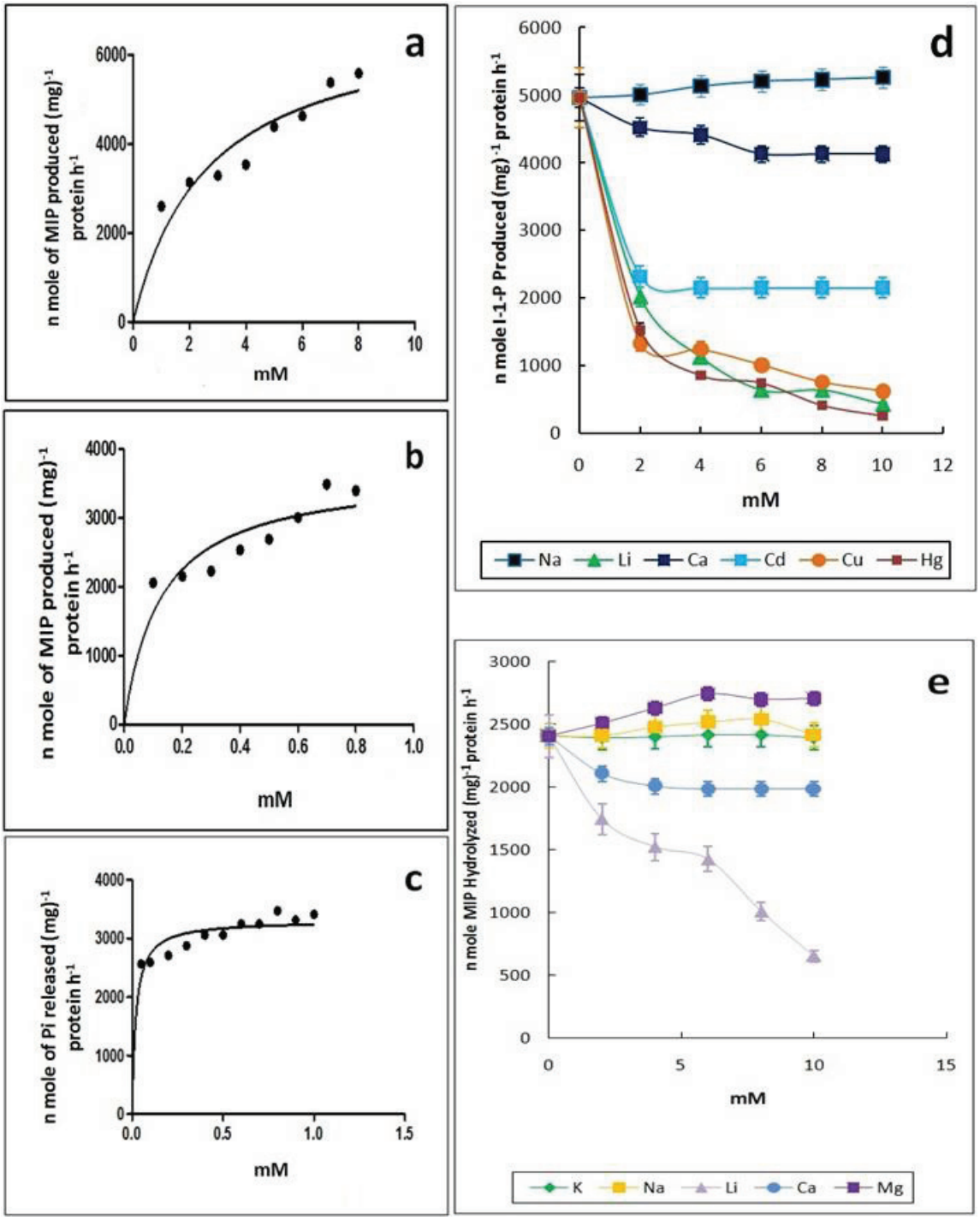

Fig. 1. Kinetic parameters and effect of different metal ion concentrations on partially purified chloroplastidial MIPS and MIPP from *Ulva lactuca.* a – determination of the Km value for glucose-6-phosphate (G-6-P) of MIPS by non-linear regression kinetics, activity is expressed as n mol MIP produced per mg protein per h; b – determination of the Km value for the coenzyme NAD (nicotinamide adenine dinucleotide) of MIPS by non-linear regression kinetics, activity is expressed as n mol MIP produced per mg protein per h; c – the effect of capricious substrate, D-*myo*-inositol-1-phosphate (D-MIP) on chloroplastidial MIPP expressed as n mol P_i released per mg protein per h; d – effect of cations on L-*myo*-inositol-1-phosphate synthase expressed as n mol-1-1-P produced per mg protein per h; e – effect of monovalent and divalent cations on MIPP activity as n mol MIP hydrolysed per mg protein per h

In case of MIPS, among the monovalent cations (at 5.0 mM conc.), the tested K^+ had trivial stimulatory role and Na^+ played an appreciably stimulatory role, while Li^+ was sturdily inhibitory (49–92%). Among the divalent cations tested (at 5.0 mM conc.), Ca^{2+} exhibited no effect, Cd^{2+} moderately inhibited (18–20 %), while Cu^{2+} (66–78%) and Hg^{2+} (86–97%) strongly inhibited the enzymatic activity (Fig. 1d). In MIPP, K^+ had slight stimulatory role, Na^+ played impartial role, but Li^+ was strongly inhibitory (46–68 %). Using the analogous concentrations of divalent cations, it was revealed that Mg^{2+} played appreciable stimulatory role and Ca^{2+} exhibited inhibitory effect at higher concentrations (Fig. 1e).

When used between the concentration range of 0 to 50 mM, EDTA exhibited a feeble inhibitory activity on chloroplastidial MIPS. However, in case of MIPP, EDTA was found to play a significant inhibitory role. The results obtained in the present investigation are indicative of the fact that MIPP activity is sensitive to metal chelators such as EDTA. Our results are in complete agreement with the results obtained in case of vascular cryptogams by previous researchers. The possible reason behind this sensitivity towards a metal chelator such as EDTA may be attributed to the presence of some already 'bound Mg^{+2}, as suggested by Patra et al. (2007).

The activity of MIPS and MIPP has been detected in almost all plant groups and these ancient enzymes are also reported to occur in different iso-forms across the plant kingdom. The results obtained in the present study not only re-establish the unique metabolic features and widespread distribution of MIPS and MIPP in the plant kingdom, but also unveils few unique metabolic features of *myo*-inositol biosynthesis and its regulation in a non-vascular cryptogam (*Ulva lactuca*) for the first time, where laboratory simulation was not provided. However, in order to understand the phylogeny of these proteins in cryptogams, a thorough search for immunologically cross-reactive material in different classes of algae or the analysis of nucleotide sequences with the aid of MIPS and MIPP gene probes may be carried out in the future.

CONCLUSIONS

Based on the main biochemical characteristics of partially purified preparation(s) of MIPS and MIPP, in the present study, it was established that salinity-induced enhancement of *myo*-inositol biosynthesis, particularly in a plant organelle such as chloroplasts, occurs in *U. lactuca* housed in its natural environment. The results obtained in the present study not only paves a new way towards a better understanding of cyclitol metabolism in salt water macrophytes, but also raises few questions regarding inositol metabolism, taking place in other genera of diverse marine algal classes (Bacillariophyceae, Xanthophyceae, Phaeophyceae, Rhodophyceae, etc.), which occur abundantly in different floras. To date, this has been substantially established in a model green algal genus, *U. lactuca*. But the biogenesis of plastids coupled with the probable biosynthesis of stress-tolerant sugar-alcohols, ROS regulation and signalling in other non-green algal groups may be different. Hence, such studies become pertinent now and from this report.

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DECLARATION OF INTEREST STATEMENT

The authors have equal contribution in this paper and there is no conflict of interest among them.

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MIOINOZITOLIO BIOSINTEZĖ JŪRINIO DUMBLIO *ULVA LACTUCA* CHLOROPLASTUOSE DRUSKŲ STRESO POVEIKYJE

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Santrauka

Straipsnyje nagrinėjama mioinozitolio biosintezė, remiantis dviejų mioinozitolio sintezės fermentų: L-mioinozitolio 1 fosfato sintazės (MIPS) ir mioinozitolio 1 fosfatazės (MIPP), tyrimais *Ulva lactuca* dumblyje, jo natūralios buveinės sąlygose. Fermentai buvo išskirti iš dumblių chloroplastų ir išvalyti, pradžioje homogenatą centrifuguojant mažu, vėliau dideliu greičiu, po to nusodinta 0–80 % amonio sulfatu, o toliau panaudojant jonų mainų chromatografijos, gelfiltravimo ir molekulinę filtravimo kolonėlę. MIPS preparato substratui buvo naudojamas D-gliukozės-6-fosfatas (G-6-P) ir koenzimas NAD, o MIPP preparato pagrindinis substratas buvo D/L-mioinozitolio 1 fosfatas. Netiesinės regresijos metodo pagalba apskaičiuota, kad G-6-P ir NAD Km aktyvumas atitinkamai lygus 2,66340 ir 0,1271 mM, o D-MIP – 0,02128 mM. Abu fermentai buvo labai aktyvūs temperatūros intervale nuo 20 iki 400° C prie optimalaus pH 7,5. Skirtingi katijonai ir organiniai jungiai turėjo kintamą poveikį abiejų fermentų aktyvumui. Nustatyta, kad *U. lactuca* laisvo mioinozitolio kiekis padidėjo proporcingai paviršiaus vandens druskingumui Čilkos lagūnoje (Odiša, Indija).