***Dictyostelium discoideum* contains three inositol monophosphatase activities with different substrate specificities and sensitivities to lithium**

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The small ion lithium, a very effective agent in the treatment of manic depressive patients, inhibits the mammalian enzyme inositol monophosphatase, which is proposed as the biological target for the effects of lithium. In this study we investigated *Dictyostelium discoideum* inositol monophosphatase activity. Partial purification of the proteins in the soluble cell fraction using anion-exchange chromatography revealed the presence of at least three enzyme activities capable of degrading inositol monophosphate isomers. The first activity was similar to the monophosphatase found in mammalian cells, as it degraded Ins(4)*P*, Ins(1)*P* and to a lesser extent Ins(3)*P*, was dependent on MgCl₂ and inhibited by LiCl in a non-competitive manner. The

second enzyme activity was specific for Ins(4)*P*; the enzyme activity was not dependent on MgCl₂ and not inhibited by LiCl. The third monophosphatase activity degraded especially Ins(3)*P*, but also Ins(4)*P* and Ins(1)*P*; increasing concentrations of MgCl₂ inhibited this enzyme activity, whereas LiCl had no effect. *In vivo*, LiCl induces a reduction of inositol levels by about 20%. In [³H]inositol-labelled cells LiCl causes a 6-fold increase in the radioactivity of [³H]Ins(1)*P*, a doubling of [³H]Ins(4)*P* and a slight decrease in the radioactivity in [³H]Ins(3)*P*. These data indicate that the biological effects of lithium in *Dictyostelium* are not due to depletion of the inositol pool by inhibition of inositol monophosphatase activity.

INTRODUCTION

Receptor-mediated hydrolysis of PtdIns(4,5)*P*₂ by the enzyme phospholipase C leads to the generation of the second messengers diacylglycerol and Ins(1,4,5)*P*₃ [1], which is metabolized by specific kinases and phosphatases to inositol. The last step in these dephosphorylation cascades, the degradation of inositol monophosphate isomers, is carried out by the enzyme inositol monophosphatase (EC 3.1.3.25) [2]. This enzyme degrades the inositol monophosphate isomers Ins(1)*P*, Ins(3)*P* and Ins(4)*P* that are formed *in vivo*. Ins(4)*P* is formed from direct dephosphorylation of Ins(1,4,5)*P*₃. Phosphorylation of the latter compound leads to the formation of Ins(1,3,4,5)*P*₄, which can also be further metabolized, eventually leading to the formation of Ins(1)*P* and Ins(3)*P*. Ins(1)*P* is also formed from phospholipase C-mediated hydrolysis of PtdIns, and Ins(3)*P* is also derived from glucose 6-phosphate in the synthesis of inositol *de novo* [2]. Mammalian inositol monophosphatase has a broad substrate specificity: as well as inositol monophosphates, a wide variety of monophosphate ester-containing compounds are degraded by the enzyme [3,4].

Mammalian inositol monophosphatase has an absolute requirement for magnesium and is non-competitively inhibited by lithium [5]. The enzyme has therefore been postulated to be the target of lithium, which is used for treatment of manic-depressive patients [6]. According to the inositol-depletion hypothesis [7], lithium depletes cells of inositol by blocking inositol monophosphatase-mediated degradation of inositol monophosphate isomers. This results in depletion of agonist-stimulated phospholipid pools and eventually in decreased formation of Ins(1,4,5)*P*₃ [8].

In the cellular slime mould *Dictyostelium discoideum*, lithium has been shown to influence development by inhibiting prespore gene expression and induction of prestalk-associated genes [9,10]. As lithium was shown to inhibit inositol monophosphatase

activity [11] and to reduce basal and cyclic AMP-stimulated Ins(1,4,5)*P*₃ levels [10], it was hypothesized also that in *Dictyostelium*, inositol depletion accounted for the effect of lithium [7,10]. However, the finding that activation of G-proteins is inhibited by lithium led to the suggestion that G-proteins might be a target of lithium [12].

To see whether inhibition of inositol monophosphatase and depletion of the inositol pool could account for the effects of lithium, we investigated the Ins(1)*P*, Ins(3)*P* and Ins(4)*P* phosphatase activities present in *Dictyostelium* soluble fraction and determined the effect of lithium *in vivo* on inositol mass and the radioactivity appearing in inositol monophosphate isomers in ³H-labelled cells.

MATERIALS AND METHODS

Materials

The Mono Q HR 5/5 column was purchased from Pharmacia. The Zorbax SAX column was obtained from Du Pont. Scintillator Plus was obtained from Packard. The nucleopore filters were from Costar. [³H]Ins(1)*P* (11.00 Ci/mmol) and [³H]Ins(4)*P* (9.9 Ci/mmol) were from NEN Du Pont. [¹⁴C]Ins(3)*P* (55 mCi/mmol) was from Amersham. Dowex X2 200/400 mesh was from Fluka, and 2,3-bisphospho-D-glycerate was obtained from Sigma. Ins(1)*P* and Ins(4)*P* were obtained from Boehringer. All other chemicals were at least analytical grade and were obtained from Merck.

Organism and culture conditions

Wild-type *Dictyostelium* cells (AX3) were grown axenically in HL5 medium [13] with the exception that the glucose concentration was 10 g/l instead of 16 g/l. Cells were harvested by centrifugation at 300 *g* for 3 min and subsequently washed with

Abbreviation used: *p*-NPP, *p*-nitrophenyl phosphate.

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PB (10 mM sodium/potassium phosphate buffer, pH 6.5). Cells were starved for 5 h at a density of 10^7 cells per ml in PB.

Preparation of soluble cell fraction

After starvation, cells were collected and lysed in TES buffer (20 mM triethanolamine, 0.5 mM EDTA and 200 mM sucrose, pH 6.5) at a density of 4×10^8 cells per ml by passage through a nucleopore filter (pore size $3 \mu\text{m}$). The lysis was checked visually afterwards, and if necessary the preparation was passed through a filter again. The sample was centrifuged for 50 min at 100 000 *g*. The supernatant was used as the soluble cell fraction.

Ion exchange chromatography

The soluble cell fraction was applied to a Mono Q ion-exchange column equilibrated with TES buffer (buffer A). Buffer B was TES buffer with 0.5 M NaCl. The column was eluted for 5 min with buffer A and then over 20 min with a linear gradient (0–40 %) of buffer B. For the next 5 min the column was eluted with a linear gradient (40–100 %) of buffer B, followed by 3 min of 100 % buffer B. The column was operated at a flow rate of 1 ml/min, fractions of 1.0 ml were collected and A_{280} was detected on-line.

Inositol monophosphatase assay

A 10 μl incubation containing about 2000 d.p.m. of radiolabelled inositol monophosphate and various concentrations of MgCl_2 , LiCl and unlabelled inositol monophosphates was incubated with 10 μl enzyme fraction obtained after Mono Q separation. Reactions were stopped by the addition of 500 μl of chloroform/methanol/fuming HCl (20:40:1 by vol.). Phase separation was induced by the addition of 200 μl of water. After brief centrifugation (2 min, 14 000 *g*), the aqueous phase was applied to 0.25 ml Dowex columns that were eluted with 6 ml of water or 6 ml of 150 mM ammonium formate/5 mM sodium tetraborate to elute inositol and inositol monophosphates respectively. After addition of 10 ml scintillator cocktail, radioactivity in the samples was determined with a Beckman liquid-scintillation counter.

General phosphatase assay

Fractions obtained after anion-exchange chromatography were incubated in a mixture containing 30 mM *p*-nitrophenylphosphate (*p*-NPP) and various concentrations of LiCl and MgCl_2 . The reaction was followed in a 96-well microtitre plate by measuring A_{415} with a Biorad microplate reader. Reactions were linear with time for at least 3 h.

Effects of lithium on inositol levels *in vivo*

Starved cells (10^7 cells in 1 ml) were incubated with 10 mM LiCl for 1 h followed by lysis in 9 % (w/v) trichloroacetic acid. The lysate was centrifuged for 5 min at 10 000 *g* and the supernatant extracted three times with water saturated ether. The mass of inositol was determined by an enzyme assay with inositol dehydrogenase and the NADH-dependent conversion of resazurin to resofurin as described previously [14].

Effects of lithium on the levels [^3H]InsP isomers *in vivo*

Starved cells (2×10^8 cells in 1 ml) were labelled with 30 μCi of [^3H]inositol by electroporation as described previously [15]. Immediately after electroporation, cells were incubated in the presence or absence of 10 mM LiCl. After 1 h, cells were washed

and lysed with perchloric acid (3.5 % w/v). After neutralization and centrifugation for 2 min at 15 000 *g*, the supernatant was analysed by HPLC on a Zorbax SAX column. For the separation of DL-[^3H]Ins(1)*P* and DL-[^3H]Ins(4)*P* the column was eluted with a gradient composed of water (solution A) and 1.2 M $(\text{NH}_4)_2\text{PO}_4$, pH 3.2 (solution B): $t = 0$, %B = 0; $t = 1$, %B = 5; $t = 4$, %B = 15; $t = 5$, %B = 20; $t = 10$, %B = 25; $t = 15$, %B = 100; $t = 19$, %B = 100 [16].

For the quantification of Ins(3)*P* and Ins(1)*P*, the HPLC column was eluted with a gradient of water (solution A) and 2M ammonium acetate, pH 3.7 (solution C): $t = 0$, %C = 0; $t = 3$, %C = 2; $t = 15$, %C = 5; $t = 16$, %C = 100. The fractions containing inositol monophosphates were pooled and desalted by freeze-drying. The content of [^3H]Ins(3)*P* was determined, after addition of about 2000 d.p.m. of [^{14}C]Ins(3)*P* as internal standard, in a reaction with *Dictyostelium* soluble fraction that has been shown to convert Ins(3)*P* to Ins₆ and not Ins(1)*P* and DL-Ins(4)*P* [17,18]. The reaction mixture (100 μl) contained 20 mM MgCl_2 , 10 mM ATP, 50 mM Tris, pH 8.0, 1 mM CaCl_2 , 1 mM EGTA, 0.25 mM 2,3-bisphospho-D-glycerate, 10 mM LiCl and 50 μl 10 000 *g* supernatant of a cell lysate from *Dictyostelium* (4×10^8 cell equivalents per ml). Of the internally added [^{14}C]Ins(3)*P*, 16 % was converted. This value was used to correct the conversion of [^3H]InsP to obtain the radioactivity in [^3H]Ins(3)*P*. The radioactivity in [^3H]Ins(1)*P* was calculated by subtracting the radioactivity in DL-[^3H]Ins(4)*P* and [^3H]Ins(3)*P* from the total radioactivity in [^3H]InsP.

RESULTS

Dictyostelium soluble cell fraction contains enzyme activities that degrade Ins(3)*P* and Ins(4)*P* [11] and Ins(1)*P* (see below). To investigate further the enzyme activities responsible for inositol monophosphate degradation, the proteins present in soluble fractions of aggregation-competent *Dictyostelium* cells were separated by anion-exchange chromatography (Figure 1, top). Figure 1 (middle) shows the activity profile obtained after incubation of the fractions with Ins(1)*P*, Ins(3)*P* and Ins(4)*P* as substrates in the presence of 5 mM MgCl_2 . The figure reveals two large activity peaks for all substrates, designated I and III. Peak I, eluting at the beginning of the NaCl gradient (fractions 11–13), degraded Ins(3)*P* much more slowly than Ins(1)*P* and Ins(4)*P*. Peak III (fractions 32–36) also degraded all three tested inositol monophosphate compounds, but in contrast Ins(3)*P* was degraded more than four times as efficiently as Ins(1)*P* and Ins(4)*P*. This indicates that monophosphatase I and III are different enzymes. In addition, fractions 14–16 immediately after peak I degrade Ins(4)*P* but not Ins(1)*P* or Ins(3)*P*, suggesting the presence of a monophosphatase activity different from monophosphatase activity I and III. This enzyme is designated monophosphatase II.

The inositol monophosphatases found in mammalian cells have a broad substrate specificity [3], including the general phosphatase substrate *p*-NPP. With this substrate, three major activity peaks were found in the anion-exchange chromatography fractions (Figure 1, bottom); the first and third peaks correspond to monophosphatases I and III respectively. At the position of monophosphatase II hardly any phosphatase activity towards *p*-NPP was found, whereas just before inositol monophosphatase III a *p*-NPP phosphatase peak was found that did not correspond to an inositol monophosphatase. These results support the notion that *Dictyostelium* contains three peaks of inositol monophosphatase activity: enzyme I and III degrade all tested inositol monophosphate isomers and *p*-NPP, whereas enzyme II is specific for Ins(4)*P*.

Figure 1 Anion-exchange chromatography of a *Dictyostelium* soluble fraction

A *Dictyostelium* soluble fraction was chromatographed on a MONO Q column. The column was eluted as described in the Materials and methods section. Top: the absorbance at 280 nm was measured on-line. Middle: the activities of the fractions towards Ins(1)*P* (▲), Ins(3)*P* (■) and Ins(4)*P* (◆) in the presence of 5 mM MgCl₂ were determined as described in the Materials and methods section. Activities are expressed as a percentage of the substrate degraded per min. Incubation times were chosen such that the reactions were under first-order kinetics. The absolute activities of the peaks varied between experiments. Bottom: the activities of the fractions towards the artificial substrate *p*-NPP incubated in the presence (□) or absence (▲) of 5 mM MgCl₂ were determined as described in the Materials and methods section. Activities are expressed as a percentage of the most active fraction. The reaction rates were linear with time during the experiment.

The monophosphatase activities indicated in Figure 1 (middle) were pooled. For enzyme I fractions 11 and 12, for enzyme II fractions 15 and 16, and for enzyme III fractions 34 and 35 were pooled. The pooled fractions were used for further investigations.

For the three inositol monophosphatase pools the MgCl₂ dependence and inhibition by LiCl were investigated with different inositol monophosphate isomers as substrate (Table 1). Enzyme I showed a markedly decreased activity in the absence of MgCl₂ for all three inositol monophosphate isomers tested. The effect was most pronounced for Ins(1)*P* and least for Ins(4)*P*, although these results did not differ significantly at $P \geq 0.05$. Increasing concentrations of LiCl inhibited this inositol monophosphatase increasingly. The effect was most pronounced in the order Ins(3)*P*, Ins(1)*P* and Ins(4)*P*. The values for Ins(1)*P* and Ins(4)*P* were not significantly different from each other, but both were significantly different from Ins(3)*P* ($P \leq 0.05$).

Enzyme II (degrading only Ins(4)*P*) was not dependent on MgCl₂ because its activity was not different in the absence or presence of MgCl₂ (Table 1). In addition, LiCl up to high concentrations had little effect on the enzyme's activity. Because fractions 11 and 12, containing enzyme I, and fractions 15 and 16, containing enzyme II, eluted close to each other, we analysed the contamination of each preparation with the other enzyme activity. Fractions 15 and 16 did not degrade Ins(1)*P* and Ins(3)*P*, indicating that this fraction was not contaminated with enzyme I. However, several observations suggested some contamination of fraction 11 and 12 with enzyme II, which is insensitive to LiCl and MgCl₂ and hydrolyses only Ins(4)*P*: first, the decreased MgCl₂ dependence of the pooled fractions 11 and 12 towards Ins(4)*P* (see above); secondly, the reduced inhibition of Ins(4)*P* degradation by LiCl (see above); and thirdly, the resistance of the pooled fractions 11 and 12 to LiCl in the absence of MgCl₂ (results not shown). From the relative activity of fractions 11 and 12 towards Ins(4)*P* in the absence of MgCl₂ and the presence of LiCl, we estimate that these fractions were contaminated with about 15–25% enzyme II. It should be noted, however, that no contamination can be detected with Ins(1)*P* or Ins(3)*P* as substrate because enzyme II does not degrade these isomers.

Enzyme III, eluting at the end of the gradient, degraded all three inositol monophosphate isomers tested. As enzyme II, this enzyme was hardly inhibited by high concentrations of LiCl (Table 1). Unlike enzymes I and II, however, this enzyme was inhibited by the presence of MgCl₂; omission of MgCl₂ from the incubation medium resulted in a 40–100% increase in inositol monophosphatase activity.

The pooled enzymes I and III were also tested with *p*-NPP as substrate. Enzyme I was inhibited 17.9% by 10 mM LiCl and 42.7% by 100 mM LiCl. In the absence of MgCl₂ this enzyme lost 79.6% of its activity compared with its activity in 5 mM MgCl₂. Enzyme III was not inhibited by 10 or 100 mM LiCl, whereas its activity in the absence of MgCl₂ was 228% of its activity in the presence of 5 mM MgCl₂. These data are consistent with those obtained with inositol monophosphates as substrates.

The mode of inhibition of enzyme I by LiCl was further investigated with Ins(1)*P* as a substrate. As Ins(1)*P* is not a substrate for enzyme II, the collected data are due solely to enzyme I. The enzyme was incubated at 5 mM MgCl₂ with different substrate concentrations in the absence and presence of 1 mM LiCl. The obtained data were analysed by non-linear fit with the program FigureP. In the absence of LiCl a K_m of 0.31 ± 0.15 mM and a V_{max} of 10.0 ± 2.5 pmol/min per μ g of protein were observed, whereas in the presence of 1 mM LiCl a K_m of 0.05 ± 0.02 mM and a V_{max} of 0.94 ± 0.10 pmol/min per μ g were determined. This means that both the K_m and V_{max} were

Table 1 Characterization of *Dictyostelium* inositol monophosphatases

Activity of inositol monophosphatase pools towards different substrates incubated in the presence or absence of 5 mM MgCl₂ and 1, 10 and 100 mM LiCl as described in the Materials and methods section. The means and S.E.M. are expressed as a percentage of the enzyme activity measured in the presence of 5 mM MgCl₂ and 0 mM LiCl. The presented data are the averages of two independent enzyme preparations assayed at least twice. Incubation times were chosen such that the reactions were under first-order kinetics.

Enzyme and substrate	[MgCl ₂] (mM) ...	5	5	5	5	0
	[LiCl] (mM) ...	0	1	10	100	0
Enzyme I						
Ins(1) <i>P</i>		100	72 ± 8	72 ± 8	33 ± 9	18 ± 4
Ins(3) <i>P</i>		100	102 ± 8	65 ± 4	12 ± 3	36 ± 3
Ins(4) <i>P</i>		100	114 ± 22	82 ± 6	49 ± 10	54 ± 16
Enzyme II						
Ins(4) <i>P</i>		100	107 ± 5	99 ± 3	70 ± 10	100 ± 4
Enzyme III						
Ins(1) <i>P</i>		100	107 ± 9	105 ± 8	90 ± 8	174 ± 9
Ins(3) <i>P</i>		100	104 ± 15	115 ± 16	95 ± 18	139 ± 19
Ins(4) <i>P</i>		100	105 ± 7	103 ± 10	97 ± 10	203 ± 8

Table 2 Effect of 10 mM LiCl on the levels of inositol and [³H]Ins*P* isomers *in vivo*

Cells were incubated in the presence or absence of 10 mM LiCl for 1 h. Inositol mass levels and radioactivity in inositol monophosphate isomers were determined as described in the Materials and methods section.

Compound	Control	10 mM LiCl	Effect (%)
Inositol (nmol/mg protein)	2.67	2.09	79
Total [³ H]Ins <i>P</i> (d.p.m.)	1068	4617	432
DL-[³ H]Ins(4) <i>P</i> (d.p.m.)	263	491	187
DL-[³ H]Ins(1) <i>P</i> (d.p.m.)	805	4126	513
[³ H]Ins(1) <i>P</i> (d.p.m.)	632	4001	633
[³ H]Ins(3) <i>P</i> (d.p.m.)	173	125	72

changed by the presence of lithium. However, the K_m/V_{max} ratio was hardly affected by the presence of lithium: it changed from 0.031 in the absence of lithium to 0.053 in the presence of 1 mM lithium. These characteristics are indicative of a non-competitive mode of inhibition by lithium [5].

For all three enzymes the kinetic constants were determined by using Ins(4)*P* as substrate as this compound is degraded by all three enzymes. Enzyme I was assayed in the presence of MgCl₂, and enzymes II and III were assayed in its absence. The K_m and V_{max} for enzyme I were 0.3 ± 0.06 mM and 6.4 ± 0.8 pmol/min per μ g of protein, respectively. For enzyme II these values were 1.3 ± 0.6 mM and 13.5 ± 5.4 pmol/min per μ g respectively. The K_m of enzyme III could not be determined because the activity was linear with substrate concentration for all concentrations tested. This means that the K_m of this enzyme is above 3 mM and the V_{max} is above 100 pmol/min per μ g.

Taken together, these results indicate that *Dictyostelium* soluble fraction contains three different enzyme activities capable of degrading inositol monophosphates. If these enzymes are active in cells, LiCl might not completely deplete inositol levels, and LiCl could have different effects on the levels of Ins(1)*P*, Ins(3)*P* and Ins(4)*P*. This was investigated by measuring the mass of inositol and the relative levels of inositol monophosphates in control and LiCl-treated cells. The results shown in Table 2 reveal that inositol levels of cells incubated with

10 mM LiCl for 1 h were still approx. 80% of the levels in control cells; longer incubation periods with LiCl did not enhance the effect (results not shown). Incubation of [³H]inositol-labelled cells with 10 mM LiCl resulted in a 4–5-fold increase in the pool of [³H]Ins*P* isomers (Table 2). Discrimination between the different inositol monophosphate isomers revealed a doubling of the amount of DL-[³H]Ins(4)*P*, whereas the amount of DL-[³H]Ins(1)*P* increased 5-fold (Table 2). A further distinction was made between [³H]Ins(1)*P* and [³H]Ins(3)*P*, using the specificity of kinases present in *Dictyostelium*, phosphorylating exclusively Ins(3)*P* to Ins*P*₆ [17,18]. This revealed that LiCl induced a 6-fold increase in the levels of [³H]Ins(1)*P*, whereas the levels of [³H]Ins(3)*P* were decreased slightly (Table 2).

DISCUSSION

In this study we demonstrate that *Dictyostelium* soluble fraction contains at least three enzyme activities capable of dephosphorylating inositol monophosphates. The first *Dictyostelium* enzyme degrades all three tested inositol monophosphates (Ins(1)*P*, Ins(3)*P* and Ins(4)*P*), and was dependent on MgCl₂. The activity of this enzyme was inhibited by LiCl in a non-competitive manner. The same properties were found when this enzyme was tested with the artificial substrate for phosphatases, *p*-NPP. These characteristics are very similar to those of mammalian inositol monophosphatase, which has a broad substrate specificity, is dependent on MgCl₂ and non-competitively inhibited by LiCl. Also the observed K_m values of this enzyme towards Ins(1)*P* and Ins(4)*P* are similar to those of the mammalian enzyme [3–5].

The second inositol monophosphatase (enzyme II) is very different from the mammalian inositol monophosphatase: this enzyme is specific for Ins(4)*P* as we did not detect any degradation of either Ins(1)*P* or Ins(3)*P* after incubation for 16 h; *p*-NPP is not degraded by enzyme II, emphasizing its high substrate specificity. Surprisingly, enzyme II is not inhibited by LiCl and is not dependent on MgCl₂. Its K_m is about three times that of enzyme I and mammalian monophosphatase.

Although the third inositol monophosphatase found in *Dictyostelium* soluble fraction degrades all three tested inositol monophosphates, it also has different characteristics from enzyme I and the mammalian inositol monophosphatase; Ins(3)*P* was degraded poorly by enzyme I but was the best substrate for

enzyme III. Furthermore enzyme III is not inhibited by LiCl and is inhibited rather than activated by MgCl_2 . The K_m of enzyme III was at least 10 times higher than that of enzyme I and the mammalian monophosphatase.

Together, these data indicate that *Dictyostelium* soluble fraction contains a mammalian-type inositol monophosphatase (enzyme I), but also two other types of inositol monophosphatase (enzymes II and III). The most interesting feature of the latter two enzymes is the lack of inhibition by LiCl. The inositol monophosphatases are hypothesized to be the cellular target of lithium, causing a cellular depletion of inositol. If the enzymes currently found in *Dictyostelium* are all active *in vivo*, it would mean that the degradation of inositol monophosphates is only partly inhibitable by lithium. Thus Ins(1)*P* is degraded mainly by the lithium-sensitive enzyme I, whereas Ins(4)*P* and Ins(3)*P* are degraded mainly by the lithium-insensitive enzymes II and III respectively.

This hypothesis corresponds well with the finding that treatment of cells with LiCl did not result in depleted inositol pools; a decrease of only 20% in the inositol mass was observed. Furthermore, treatment of [^3H]inositol-labelled cells with 10 mM LiCl resulted in a doubling of the radioactivity in DL-Ins(4)*P*. Because in *Dictyostelium* there are no indications for the presence of Ins(6)*P*, we assume that it is Ins(4)*P*. The radioactivity in DL-Ins(1)*P*, however, increased 5-fold, which was totally due to an increase in Ins(1)*P*; the levels of Ins(3)*P* were even slightly decreased.

In conclusion, *Dictyostelium* lysates contain at least three enzyme activities degrading inositol monophosphates, of which two are lithium-insensitive. If these enzymes are active in cells, it is expected that lithium cannot inhibit the degradation of all inositol monophosphate isomers to the same extent. The observation that lithium induces a potent increase in Ins(1)*P*, a minor increase in Ins(4)*P* and a small decrease in Ins(3)*P* supports the hypothesis of multiple monophosphatases acting *in vivo*. Although lithium induces a small decrease in inositol levels in *Dictyostelium*, it is possible that the effects of lithium in this

organism are not mediated by depletion of the inositol pool. It was previously shown that lithium inhibits the activation of G-proteins in *Dictyostelium* [12]. If lithium does not act via depletion of the inositol pool, G-proteins could be the targets that are responsible for the observed effects of lithium, as they are involved in many cellular processes.

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