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

Peter VAN DIJKEN, Jan C. T. BERGSMA, Hoebert S. HIEMSTRA, Berber DE VRIES, Jeroen VAN DER KAAY and Peter J. M. VAN HAASSTERT

Department of Biochemistry, University of Groningen, Nijenborgh 4, 9747 AG, Groningen, The Netherlands

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)P₂ by the enzyme phospholipase C leads to the generation of the second messengers diacylglycerol and Ins(1,4,5)P₃ [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)P₃. Phosphorylation of the latter compound leads to the formation of Ins(1,3,4,5)P₄, 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 monophosphate-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)P₃ [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)P₃ 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 nuclease 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. *[^3C]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.

* To whom correspondence should be addressed.
Immediately after electroporation, cells were incubated in the resazurin to resofurin as described previously [14]. Inositol was determined by an enzyme assay with inositol 1m l

 effects of lithium on the levels [3H]Ins

 starved cells (10

 in vivo

 effects of lithium on the levels [3H]InsP isomers in vivo

 effects of lithium on levels [3H]InsP isomers in vivo

 preparation of soluble cell fraction

 ion exchange chromatography

 inositol monophosphatase assay

 general phosphatase assay

 effects of lithium on inositol levels

 effects of lithium on inositol levels
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 ≥ 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 ≤ 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 Kₘ of 0.31 ± 0.15 mM and a Vₘₐₓ of 10.0 ± 2.5 pmol/min per µg of protein were observed, whereas in the presence of 1 mM LiCl a Kₘ of 0.05 ± 0.02 mM and a Vₘₐₓ of 0.94 ± 0.10 pmol/min per µg were determined. This means that both the Kₘ and Vₘₐₓ were

---

**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.
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.

<table>
<thead>
<tr>
<th>Enzyme and substrate</th>
<th>[MgCl₂] (mM) × 10⁻³</th>
<th>[LiCl] (mM)</th>
<th>0</th>
<th>1</th>
<th>10</th>
<th>100</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enzyme I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ins(1)P</td>
<td>100</td>
<td>72±0.8</td>
<td>72±0.8</td>
<td>33±0.9</td>
<td>18±0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ins(3)P</td>
<td>100</td>
<td>102±0.8</td>
<td>65±0.4</td>
<td>12±0.3</td>
<td>36±0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ins(4)P</td>
<td>100</td>
<td>114±0.22</td>
<td>82±0.6</td>
<td>49±0.10</td>
<td>54±0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enzyme II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ins(4)P</td>
<td>100</td>
<td>107±0.5</td>
<td>99±0.3</td>
<td>70±0.10</td>
<td>100±0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enzyme III</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ins(1)P</td>
<td>100</td>
<td>107±0.9</td>
<td>105±0.8</td>
<td>90±0.8</td>
<td>174±0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ins(3)P</td>
<td>100</td>
<td>104±15</td>
<td>115±16</td>
<td>95±18</td>
<td>139±19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ins(4)P</td>
<td>100</td>
<td>105±7</td>
<td>103±10</td>
<td>97±10</td>
<td>203±8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Effect of 10 mM LiCl on the levels of inositol and [³H]InsP 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.

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

DISCUSSION

In this study we demonstrate that Dictyostelium soluable fraction contains at least three enzyme activities capable of dephosphorylating inositol monophosphates. The first Dictyostelium enzyme degrades all three tested inositol mono-phosphates (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, and is dependent on MgCl₂ and non-competitively inhibited by LiCl. Also the observed Kₘ 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 Kₘ is about three times that of enzyme I and mammalian monophosphatase.

Although the third inositol monophosphatase found in Dictyostelium soluable 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

changed by the presence of lithium. However, the Kₘ/Vₘₐₓ 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 Kₘ and Vₘₐₓ for enzyme I were 0.3 ± 0.06 mM and 6.4 ± 0.8 pmol/min per µg of protein, respectively. For enzyme II these values were 1.3 ± 0.6 mM and 13.5 ± 5.4 pmol/min per µg, respectively. The Kₘ of enzyme III could not be determined because the activity was linear with substrate concentration for all concentrations tested. This means that the Kₘ of this enzyme is above 3 mM and the Vₘₐₓ is above 100 pmol/min per µg.

Taken together, these results indicate that Dictyostelium soluable 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]InsP 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 Ins₃P [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).
enzyme III. Furthermore enzyme III is not inhibited by LiCl and is inhibited rather than activated by MgCl₂. The Kᵢ₅₀ 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 inhabitable 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.

This study was supported by the Foundation for Life Sciences (SLW), which is subsidized by the Netherlands Organization for Scientific Research (NWO).

REFERENCES