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Two dephosphorylation pathways of inositol 1,4,5-trisphosphate in homogenates of the cellular slime mould Dictyostelium discoideum

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**Dictyostelium discoideum** homogenates contain phosphatase activity which rapidly dephosphorylates Ins(1,4,5)P3 (d-myo-inositol 1,4,5-trisphosphate) to Ins (myo-inositol). When assayed in Mg⁺⁺, Ins(1,4,5)P3 is dephosphorylated by the soluble *Dictyostelium* cell fraction to 20% Ins(1,4)P2 (d-myo-inositol 1,4-bisphosphate) and 80% Ins(4,5)P2 (d-myo-inositol 4,5-bisphosphate). In the particulate fraction Ins(1,4,5)P3 5-phosphatase is relatively more active than the Ins(1,4)P2 1-phosphatase. CaCl₂ can replace MgCl₂ only for the Ins(1,4,5)P3 5-phosphatase activity. Ins(1,4)P2 and Ins(4,5)P2 are both further dephosphorylated to Ins4P (d-myo-inositol 4-monophosphate), and ultimately to Ins. Li⁺ ions inhibit Ins(1,4,5)P3 1-phosphatase, Ins(1,4)P2 1-phosphatase, Ins4P phosphatase and l-InsP (l-myo-inositol 1-monophosphate) phosphatase activities; Ins(1,4,5)P3 1-phosphatase is 10-fold more sensitive to Li⁺ (half-maximal inhibition at about 0.25 mM) than are the other phosphatases (half-maximal inhibition at about 2.5 mM). Ins(1,4,5)P3 5-phosphatase activity is potentely inhibited by 2,3-bisphosphoglycerate (half-maximal inhibition at 3 μM). Furthermore, 2,3-bisphosphoglycerate also inhibits dephosphorylation of Ins(4,5)P2. These characteristics point to higher organisms. The presence of an hitherto undescribed Ins(1,4,5)P3 1-phosphatase, however, causes the formation of a different inositol bisphosphatase isomer [Ins(4,5)P2] from that found in higher organisms [Ins(1,4)P2]. The high sensitivity of some of these phosphatases for Li⁺ suggests that they may be the targets for Li⁺ during the alteration of cell pattern by Li⁺ in *Dictyostelium*.

INTRODUCTION

The pivotal role of Ins(1,4,5)P3 as the second messenger for receptor-mediated Ca²⁺ mobilization has been firmly established in a wide variety of systems (reviews: Downes & Michell, 1985; Berridge, 1987). In the best studied mammalian systems, such as human erythrocytes, platelets, rat brain, liver, pancreas and parotid gland, the Ins(1,4,5)P3 response is attenuated by a specific phosphatase which removes the phosphate from the 5-position to yield Ins(1,4)P2 (Downes et al., 1982; Storey et al., 1984; Connolly et al., 1985; Erneux et al., 1986; Shears et al., 1987). Ins(1,4)P2 is then further dephosphorylated to Ins4P in rat liver and brain and calf brain (Delvaux et al., 1987a; Ackermann et al., 1987; Inhorn et al., 1987; Ragan et al., 1988), and finally to Ins. The Ins formed in this way can then be re-used for the synthesis of inositol phospholipids, thus closing the cyclic metabolic pathway characteristic for this signalling system.

Dephosphorylation of Ins(1,4)P2 and InsP has been shown to be sensitive to Li⁺ ions (Hallecker & Sherman, 1980; Storey et al., 1984; Takimoto et al., 1985; Delvaux et al., 1987a,b; Gee et al., 1988), and it has been suggested that the pharmacological effect of Li⁺ as a drug against manic–depressive illness might be due to inhibition of these enzymes by Li⁺ (Drummond, 1987).

In the cellular slime mould *Dictyostelium discoideum*, which is frequently used as a model for studying signal transduction and differentiation, a similar second-messenger function has been proposed for Ins(1,4,5)P3; Ins(1,4,5)P3 can elicit Ca²⁺ release from non-mitochondrial Ca²⁺ stores in saponin-permeabilized *Dictyostelium* cells (Europe-Finner & Newell, 1986), and more recently it was shown that the chemoattractant cyclic AMP can trigger the accumulation of intracellular InsP₂ in vivo (Europe-Finner & Newell, 1987). Furthermore, Li⁺ ions can alter the pattern in *Dictyostelium* slugs and direct differentiation to the stalk pathway (Maeda, 1970; Sakai, 1973; Van Lookeren Campagne et al., 1988).

Very little is known about the enzymes involved in the turnover of inositol phospholipids and inositol phosphates in *D. discoideum*. The only enzymes that have been described are the CDP-diacylglycerol:inositol phosphatidyltransferase and the Mn²⁺-catalysed phosphatidylinositol:myo-inositol exchange activity (Machon et al., 1980), a kinase which phosphorylates phosphatidylinositol to phosphatidylinositol 4-phosphate (Varela et al., 1987) and a kinase which phos-

Abbreviations used: Ins, myo-inositol; Ins1P, d-myo-inositol 1-phosphate; l-Ins1P, L-myo-inositol 1-phosphate; Ins4P, d-myo-inositol 4-phosphate; Ins(1,4)P₂, d-myo-inositol 1,4-bisphosphate; Ins(4,5)P₂, d-myo-inositol 4,5-bisphosphate; Ins(1,5)P₂, d-myo-inositol 1,5-bisphosphate; Ins(1,4,5)P₃, d-myo-inositol 1,4,5-trisphosphate; InsP, InsP₂ and InsP₃, d-myo-inositol phosphates without specification of the phosphate position(s).

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phorylates diacylglycerol to phosphatidic acid (Jimenez et al., 1988). Phosphatidilinositol 4-phosphate kinase is also present in Dictyostelium (M. M. Van Lookeren Campagne, unpublished work), but no phospholipase C activity has been demonstrated up to now (Irvine et al., 1980). It was not known whether Dictyostelium cells have enzymes which dephosphorylate Ins(1,4,5)P₃. Here we report that Ins(1,4,5)P₃ can be dephosphorylated to Ins in D. discoideum homogenates. Furthermore, we show that this dephosphorylation can occur by two different routes, with as intermediates either Ins(1,4)P₂ or Ins(4,5)P₂, which are both dephosphorylated, through Ins4P, to Ins.

MATERIALS AND METHODS

Materials

[2-³H]Ins(1,4,5)P₃ (1.0 Ci/mmol) and l-¹⁴C]Ins1P (55 mCi/mmol) were from Amersham International. [2-³H]Ins(1,4)P₂ (2.0 Ci/mmol), [4,5-³²P]Ins(1,4,5)P₃ (130 Ci/mmol), [2-³H]Ins1P (5.4 Ci/mmol) and [2-³H]-Ins4P (1.5 Ci/mmol) were from New England Nuclear. Dowex 1 (200–400 mesh) and 2,3-bisphosphoglycerate were from Sigma. The h.p.l.c. columns were from Waters (µBondapak NH₂; 30 cm × 0.39 cm), Whatman (Partisil SAX; 25 cm × 0.49 cm) and Chrompack (LiChrosorb 10RP18; 25 cm × 0.49 cm).

Organism and culture conditions

Dictyostelium discoideum strain NC-4(H) was grown in association with Escherichia coli 281 on glucose/peptone agar as described previously (Van Lookeren Campagne et al., 1986). Amoebae were harvested in 10 mM-phosphate buffer, pH 6.5, and freed from bacteria by repeated centrifugation. Cells were then plated on non-nutrient agar plates at a density of 10⁷ cells/cm² and incubated overnight at 6°C to induce full aggregation-competence (Konijn, 1970).

Homogenate

Aggregation-competent cells were harvested in 10 mM-phosphate buffer, pH 6.5, washed once in ice-cold buffer A (20 mM-Hepes/NaOH, 0.5 mM-EDTA, 200 mM-sucrose, pH 7.0), and resuspended to 2 × 10⁶ cells/ml in the same buffer. Homogenates were made by passing the cells through a Nucleopore filter (3 μm pore size) (Das & Henderson, 1983). Lysates were then centrifuged for 3 min at 10000 g. The particulate cell fraction was prepared by washing the pellet once in buffer A and resuspending it in the same buffer to the original volume of the homogenate. The soluble fraction was prepared by recentrifuging the 10000 g supernatant for 5 min in a Beckman Airfuge at 150000 g. Soluble and particulate fractions thus obtained were immediately used for the phosphatase assay.

Phosphatase assay

Dephosphorylation of [2-³H]Ins(1,4,5)P₃, [4,5-³²P]Ins(1,4,5)P₃, [2-³H]Ins(1,4)P₂, [2-³H]Ins(4,5)P₂, [4,5-³²P]Ins(4,5)P₂ and/or l-¹⁴C]Ins1P was assayed in buffer A, in the presence of either 5 mM-MgCl₂ or 2.5 mM-CaCl₂, at 22°C. Incubations were started by adding 5 μl of either the soluble or particulate fraction of the Dictyostelium homogenate to 15 μl assay mixture, containing 1000–3000 c.p.m. of radio-labelled substrate. Reactions were stopped after 5–30 min by adding 0.5 ml of chloroform/methanol/conc. HCl (20:40:1, by vol.). Phases were separated by adding 200 μl of water. After vigorous shaking and centrifugation (1 min, 10000 g), the aqueous phase was applied to 0.5 ml Dowex-1 anion-exchange columns (formate form). The different reaction products were separated by stepwise elution with: (1) 10 ml of water (Ins); (2) 10 ml of 150 mM-ammonium formate/5 mM-Na₂HPO₄ (InsP and P₃); (3) 10 ml of 300 mM-ammonium formate/100 mM-formic acid (InsP₂); and (4) 10 ml of 750 mM-ammonium formate/100 mM-formic acid (InsP). Radioactivity of the fractions was measured by liquid-scintillation counting after adding 13 ml of Instagel (Packard).

When it was necessary to separate Ins4P from Ins1P and P₃, reaction products (usually with the internal standard of l-¹⁴C]Ins1P) were separated by anion-exchange h.p.l.c. as described in the Figure legends. Ins(1,4,5)P₃ 5-phosphatase activity from human erythocyte membranes was assayed as described previously (Erneux et al., 1986). Enzyme activities were approximately linear with time and enzyme concentrations, provided that not more than about 25% of the substrate was utilized. The S.D. of the phosphatase assay was about 10%. Experiments were performed at least three times with similar results; the analysis of the InsP isomers by h.p.l.c. was performed twice with identical results.

Preparation and purification of Ins(4,5)P₃

Aggregation-competent Dictyostelium cells were lysed in buffer B (50 mM-Tris/HCl, pH 7.2, 10% (v/v) glycerol, 10 mM-dithiothreitol, leupeptin (6.5 μg/ml), 100 mM-phenylmethanesulphonyl fluoride, soya-bean trypsin inhibitor (50 μg/ml) and 5 mM-benzamidine). The high-speed supernatant from 3 × 10⁶ cells was chromatographed on a DEAE-cellulose column (10 ml; 8 cm × 1.3 cm), which was equilibrated and eluted in buffer B. The Ins(1,4,5)P₃ 5-phosphatase activity binds to the column, whereas the Ins(1,4,5)P₃ 1-phosphatase is eluted from the column between 1.5 and 2 column vol. (P. J. M. Van Haastert & E. Rovers, unpublished work).

A mixture of ³²P- and ³H-labelled Ins(4,5)P₃ was prepared in an incubation (100 μl) containing 50 nCi of [4,5-³²P]Ins(1,4,5)P₃, 100 nCi of [2-³H]Ins(1,4,5)P₃, 5 mM-MgCl₂, buffer A and 40 μl of enzyme from the DEAE-cellulose column. After 60 min the incubation was terminated by the addition of 100 μl of 0.1 M-tritylammonium phosphate, pH 6.5. The sample was centrifuged immediately for 5 min at 10000 g, and the supernatant was chromatographed by h.p.l.c. on a reversed-phase LiChrosorb 10RP18 column, which was eluted isocratically with 1.5 mM-tritylammonium phosphate / 4.5 mM-tritylammonium formate / 16% (v/v) methanol, pH 6.5, at a flow rate of 1.2 ml/min. Fractions of volume 0.6 ml were collected; the radioactivity of 6 μl samples was determined by using a dual-label program. Peak fractions were combined and concentrated to dryness under reduced pressure at 10°C. [³H]Ins(4,5)P₃ was prepared in parallel from 100 nCi of [³H]Ins(1,4,5)P₃.
Table 1. Relative Ins(1,4,5)P_3 phosphatase activity in soluble and particulate cell fractions measured under different conditions

<table>
<thead>
<tr>
<th>Assay with:</th>
<th>No addition</th>
<th>25 mM LiCl</th>
<th>0.25 mM-2,3-Bisphosphoglycerate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mM-MgCl_2</td>
<td>100</td>
<td>21</td>
<td>82</td>
</tr>
<tr>
<td>2.5 mM-CaCl_2</td>
<td>19</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>Particulate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mM-MgCl_2</td>
<td>21</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>2.5 mM-CaCl_2</td>
<td>11</td>
<td>10</td>
<td>1</td>
</tr>
</tbody>
</table>

RESULTS

General properties of Ins(1,4,5)P_3 dephosphorylation in Dictyostelium

Ins(1,4,5)P_3 can be rapidly dephosphorylated by a D. discoideum homogenate to InsP_2. Activity is optimal at pH 7.0 and 5 mM-MgCl_2 (results not shown) and is located predominantly in the soluble cell fraction (Table 1). LiCl and 2,3-bisphosphoglycerate, which are known inhibitors of phospho-inositol phosphatases (Downes et al., 1982; Storey et al., 1984; Delvaux et al., 1987a) have differential potencies in the soluble and particulate fractions. Li^+ being a more potent inhibitor of the soluble Ins(1,4,5)P_3 phosphatase activity and 2,3-bisphosphoglycerate being more potent on the particulate activity (Table 1). The particulate enzyme is probably not located on the cell surface, because intact cells express little enzyme activity (results not shown).

CaCl_2 (2.5 mM) can replace MgCl_2 to a certain extent, more so in the particulate fraction (to about 50%) than in the soluble fraction (to about 20%). Furthermore, replacement of Ca^{2+} for Mg^{2+} changes the sensitivity towards Li^+ and 2,3-bisphosphoglycerate, Li^+ becoming ineffective and 2,3-bisphosphoglycerate almost completely inhibiting all phosphatase activity (Table 1).

Identification of the first phosphate group removed by Ins(1,4,5)P_3 phosphatase

The studies with the Ins(1,4,5)P_3 phosphatase inhibitors Li^+ and 2,3-bisphosphoglycerate suggest that in 5 mM-Mg^{2+} Ins(1,4,5)P_3 is dephosphorylated by a route different from that in 2.5 mM-Ca^{2+}. To see which phosphate group is removed first under the different conditions, we measured the hydrolysis of a mixture of \([4,5\text{-}^{32}P]\text{Ins}(1,4,5)P_3\) and \([2\text{-}^{3}H]\text{Ins}(1,4,5)P_3\). The distribution of \(^{32}P\) label between the 4- and 5-phosphates of the commercial \([4,5\text{-}^{32}P]\text{Ins}(1,4,5)P_3\) was 12% at the 4- and 88% at the 5-position (as determined with specific 5-phosphatase in human erythrocyte membranes; Downes et al., 1982). After incubation of this \([4,5\text{-}^{32}P]\text{Ins}(1,4,5)P_3\) and \([2\text{-}^{3}H]\text{Ins}(1,4,5)P_3\) mixture with the D. discoideum soluble cell fraction, and subsequent fractionation of the inositol phosphates formed on Dowex columns, the ratio of \(^{32}P\) label recovered with respect to the \(^{3}H\) label in the InsP_2 column fraction was determined. As the \([4,5\text{-}^{32}P]\text{Ins}(1,4,5)P_3\) is labelled predominantly in the 5-position, a low \(^{32}P\text{:}^{3}H\) ratio in InsP_2 indicates 5-phosphatase activity, whereas a high \(^{32}P\text{:}^{3}H\) ratio in InsP_2 indicates 1- and/or 4-phosphatase activity.

Table 2 shows that the \(^{32}P\text{:}^{3}H\) ratio in InsP_2 is 82%, of the original value in the Ins(1,4,5)P_3 substrate, when dephosphorylation is assayed in 5 mM-Mg^{2+}. In the presence of 2,3-bisphosphoglycerate the ratio even approaches the original ratio in Ins(1,4,5)P_3 (92%). This suggests that, in 5 mM-Mg^{2+}, Ins(1,4,5)P_3 is dephosphorylated predominantly at the 1- and/or the 4-position, thus yielding Ins(1,5)P_2 and/or Ins(4,5)P_2. In contrast, the Li^+-insensitive part of the activity in Mg^{2+} dephosphorylates Ins(1,4,5)P_3 at the 5-position, as the \(^{32}P\text{:}^{3}H\) ratio is similar to that found for the 5-phosphatase from erythrocyte membrane (Table 2). The same is the case for the activity measured in Ca^{2+}.

From this we conclude that in the soluble fraction of Dictyostelium homogenates, assayed for Ins(1,4,5)P_3 phosphatase in Mg^{2+}, about 80% of the activity is accounted for by either a 1- and/or a 4-phosphatase, which is sensitive to Li^+, and about 20% of the activity.
by a 5-phosphatase, which is sensitive to 2,3-bisphosphoglycerate. Ca²⁺ can only replace Mg²⁺ for the 5-phosphatase. In the particulate fraction the 5-phosphatase and 1- and/or 4-phosphatase are about equally active.

Analysis of ³²P/³⁵H ratios does not give a clear-cut answer about whether the Li⁺-sensitive Ins(1,4,5)P₃ phosphatase activity dephosphorylates Ins(1,4,5)P₃ at the 1- or at the 4-position, as the fraction of ³²P label in the 4-position is too small for accurate analysis. As we do know that the InsP₁ thus formed still contains the 5-phosphate, the two possible isomers of this InsP₁ are Ins(4,5)P₂ or Ins(1,5)P₂, and it is therefore termed Ins(x,5)P₂.

Dephosphorylation of InsP₂

The two distinct pathways for the first step in InsP₂ dephosphorylation, measured in 5 mM-MgCl₂, have been shown above to yield Ins(x,5)P₂ (80%) and Ins(1,4)P₂ (20%). We used [²⁻H]Ins(1,4)P₂ as a substrate to characterize the second dephosphorylation step of the latter pathway. H.p.l.c. analysis of the products formed after incubating Ins(1,4)P₂ with the Dictyostelium soluble cell fraction shows that the InsP formed from Ins(1,4)P₂ is exclusively Ins4P (Fig. 1).

To study the dephosphorylation of Ins(x,5)P₂ to InsP₁, we synthesized and purified a ³²P/³⁵H-labelled mixture of Ins(x,5)P₂ from [4,5-³²P]Ins(1,4,5)P₃ and [³²³⁵H]Ins(1,4,5)P₃ (see the Materials and methods section and Fig. 2). The ³²P/³⁵H ratio of the Ins(1,4,5)P₃ substrate was 0.60; the ratio in the Ins(x,5)P₂ product was 0.61. This purified ³²P/³⁵H mixture of Ins(x,5)P₂ was incubated with Dictyostelium soluble cell fraction, and the dephosphorylation products were subsequently analysed by h.p.l.c. (Fig. 3). The InsP₁ formed is co-eluted with Ins₄P, is not co-eluted with Ins₁P, and has a low ³²P/³⁵H ratio (0.05) compared with the synthesized Ins(x,5)P₂ substrate (0.61), indicating that the 5-phosphate has been removed. From this we infer that the InsP₁ formed is Ins₄P, and thus the synthesized Ins(x,5)P₂ must have been Ins(4,5)P₃ [and not Ins(1,5)P₂]. In summary, we can conclude that Ins(1,4,5)P₃ is dephosphorylated by Dictyostelium soluble cell fraction, through Ins(1,4)P₂ and predominantly Ins(4,5)P₃, to Ins₄P.
Dephosphorylation of inositol trisphosphate in Dictyostelium

Fig. 3. H.p.l.c. analysis of the InsP isomer(s) formed after dephosphorylation of a $^{32}$P/$^3$H-labelled mixture of Ins(x,5)$_P$, synthesized from $[4,5-^{32}$P]$^{3}$Ins(1,4,5)$_P$ and $[2-^{3}$H]$^{3}$Ins(1,4,5)$_P$.

H.p.l.c.-purified $[3^{32}$P,$^3$H]$^{3}$Ins(x,5)$_P$ (see Fig. 2) was incubated with Dictyostelium soluble cell fraction for 60 min in the presence of 5 mM-MgCl$_2$. The incubation was terminated by adding 0.5 ml of ice-cold 150 mM-ammonium acetate/acetic acid (pH 4.0) and immediate filtration on a Centricon TM microconcentrator. The reaction products were separated on a Partisil SAX column by isocratic elution with 150 mM-ammonium acetate/acetic acid, pH 4.0. Fractions were collected every 1 min (flow rate 1.5 ml/min), and radioactivity was determined by liquid-scintillation spectrometry. The $^{32}$P/$^3$H ratio in the InsP peak is low (0.05, relative to 0.61 in substrate), indicating that it does not contain a 5-phosphate group. Co-elution with authentic Ins4P and absence of a 5-phosphate in the InsP identifies the product as Ins4P, and the Ins(x,5)$_P$ substrate as Ins(4,5)$_P$, $^{32}$P radioactivity; $^{3}$H radioactivity.

Inhibition of Ins(1,4,5)$_P$ phosphatase by Li$^+$

As shown in Table 1, the inhibitor-sensitivity of Ins(1,4,5)$_P$ dephosphorylation is rather complex. We have therefore studied the sensitivity of the different dephosphorylation reactions in more detail. In mammalian tissues, Li$^+$ does not affect the 5-phosphatase activity acting on Ins(1,4,5)$_P$ (Downes et al., 1982; Storey et al., 1984; Connolly et al., 1985; Erneux et al., 1986), but is a potent uncompetitive inhibitor of 1- and 4-phosphatase activities acting on Ins(1,4)$_P$, Ins4P and Ins1P (Hallecker & Sherman, 1980; Inhorn & Majerus, 1987; Gee et al., 1988). As shown in Fig. 4, Li$^+$ effectively inhibits Ins(1,4,5)$_P$ phosphatase activity to a maximum of about 80%, if measured in 5 mM-Mg$^{2+}$ with 0.25 mM-Ins(1,4,5)$_P$. Half-maximal inhibition of this activity occurs at about 0.25 mM-LiCl. When phosphatase activity was measured in Ca$^{2+}$, Li$^+$ had no effect (Fig. 4).

Fig. 4. Effect of Li$^+$ on Ins(1,4,5)$_P$ phosphatase activity in the soluble cell fraction of Dictyostelium homogenates.

Dephosphorylation of $[2-^{3}$H]$^{3}$Ins(1,4,5)$_P$ (5 nCi; 0.25 $\mu$m) was assayed in the presence of different LiCl concentrations as described in the Materials and methods section, with either 5 mM-MgCl$_2$ (●) or 2.5 mM-CaCl$_2$ (○). The phosphatase activity data are expressed as percentages of the control without LiCl.

Inhibition of Ins(1,4,5)$_P$ phosphatase by 2,3-bisphosphoglycerate

2,3-Bisphosphoglycerate is a potent competitive inhibitor of 5-phosphatase activities from erythrocyte membranes (Downes et al., 1982) and soluble and particulate rat brain fraction (Delvaux et al., 1987a). Furthermore, concentrations of up to 1 mM have no effect on Ins(1,4)$_P$ and Ins1P phosphatases (Delvaux et al., 1987a).

In the Dictyostelium soluble cell fraction 2,3-bisphosphoglycerate inhibits the Ins(1,4,5)$_P$ phosphatase activity biphasically, when measured with 0.25 mM-Ins(1,4,5)$_P$ in 5 mM-Mg$^{2+}$ (Fig. 5). Under these conditions, about 20% of the activity is inhibited with high sensitivity, whereas the remaining 80% is inhibited only at high concentrations (above 0.5 mM) of 2,3-bisphosphoglycerate. When measured in Ca$^{2+}$, however, all the activity can be inhibited with high sensitivity, half-maximal inhibition occurring at about 3 $\mu$m (Fig. 5).

The combination of the effects of Li$^+$ and 2,3-bisphosphoglycerate on the Dictyostelium Ins(1,4,5)$_P$ phosphatase activity (assuming that the inhibitor-sensitivities of the Dictyostelium and rat brain enzymes are similar) supplements the evidence presented above that in Mg$^{2+}$ Ins(1,4,5)$_P$ is dephosphorylated by two enzymes: 20% of the activity is due to a 5-phosphatase, as this 20% of the activity is insensitive to Li$^+$ and highly sensitive to 2,3-bisphosphoglycerate, and 80% of the activity is due to a 1-phosphatase, as this activity is less sensitive to 2,3-bisphosphoglycerate and highly sensitive to Li$^+$. Ca$^{2+}$ apparently can only replace Mg$^{2+}$ in that part of the activity which is sensitive to 2,3-bisphosphoglycerate, so in Ca$^{2+}$ all activity is apparently due to a 5-phosphatase which has some similarities to the 5-phosphatase from higher organisms.
Fig. 5. Effect of 2,3-bisphosphoglycerate on Ins(1,4,5)P$_2$ phosphatase activity in the soluble cell fraction of Dictyostelium homogenates

Dephosphorylation of [2-3H]Ins(1,4,5)P$_2$ (5 nCi; 0.25 μM) was assayed in the presence of different 2,3-bisphosphoglycerate concentrations as described in the Materials and methods section, with either 5 mM-MgCl$_2$ (●) or 2.5 mM-CaCl$_2$ (○). The phosphatase activity data are expressed as percentages of the control without 2,3-bisphosphoglycerate.

Fig. 6. Effect of Li$^+$ on Ins(1,4)P$_2$ 1-phosphatase activity in the soluble cell fraction of Dictyostelium homogenates

Dephosphorylation of [2-3H]Ins(1,4)P$_2$ (5 nCi; 0.125 μM) was assayed in the presence of different LiCl concentrations as described in the Materials and methods section. The phosphatase activity data are expressed as percentages of the control without LiCl. The inset shows a Dixon plot of the same data.

Fig. 7. Effect of Li$^+$ on 1-Ins1P phosphatase activity in the soluble cell fraction of Dictyostelium homogenates

Dephosphorylation of [1-14C]Ins1P (6.3 nCi; 5.7 μM) was assayed as described for InsP$_2$ phosphatase in Fig. 6.

Sensitivity of InsP$_2$ dephosphorylation to Li$^+$ and 2,3-bisphosphoglycerate

Dephosphorylation of Ins(1,4)P$_2$ to Ins4P has been shown to be Li$^+$-sensitive and 2,3-bisphosphoglycerate-insensitive in rat brain (Inhorn et al., 1987; Delvaux et al., 1987a). The same is the case for [2-3H]Ins(1,4)P$_2$ dephosphorylation in Dictyostelium; Li$^+$ inhibits the 1-phosphatase activity with half-maximal inhibition at about 2.5 mM (Fig. 6), and 0.25 mM-2,3-bisphosphoglycerate has no effect on this activity (results not shown).

To measure the sensitivities of Ins(4,5)P$_2$ dephosphorylation to Li$^+$ and 2,3-bisphosphoglycerate, we synthesized and purified [2-3H]Ins(4,5)P$_2$ from [2-3H]Ins(1,4,5)P$_2$ (see the Materials and methods section) and incubated the compound with the high-speed supernatant from Dictyostelium: 25 mM-LiCl inhibits the dephosphorylation of [2-3H]Ins(4,5)P$_2$ by only 7%, whereas 0.25 mM-2,3-bisphosphoglycerate inhibits the dephosphorylation by 76% (results not shown).

Li$^+$-sensitivity of Ins4P and 1-Ins1P dephosphorylation

myo-Inositol monophosphates can be derived from two different sources: (1) 1-Ins1P formed from isomerization of D-glucose 6-phosphate catalysed by 1-myoinositol 1-phosphate synthase, which is required for Ins synthesis de novo, and (2) InsP in the D-conformation, formed through the action of phospholipase C on phosphatidylinositol, or through dephosphorylation of the D-myoinositol polyphosphates. In high organisms, InsP is dephosphorylated by a phosphatase which is not very specific; the enzyme can hydrolyse all InsP isomers with an equatorial phosphate group, as well as 2'-AMP and (−)-chiro-Ins(3)P but not Ins(1,4,5)P$_2$] and does not discriminate between the two enantiomeric conformations of Ins1P (Eisenberg, 1967; Hallcker & Sherman, 1980; Ackermann et al., 1987). Furthermore, Li$^+$ is a potent inhibitor of InsP phosphatase activities (Naccarato et al., 1974; Hallcker & Sherman, 1980; Sherman et al., 1984; Ackermann et al., 1987; Delvaux et al., 1987b; Gee et al., 1988).
In *Dictyostelium* homogenates, 1-Ins1P and Ins4P are dephosphorylated under conditions similar to those described for the above systems. Li⁺ inhibits the dephosphorylation of 1-[U-14C]Ins1P in the soluble fraction of these homogenates, with half-maximal inhibition at about 2.5 mM (Fig 7). Furthermore, when [2-3H]Ins(1,4)P₂ is incubated with this cell fraction until almost no substrate is left, subsequent addition of Li⁺ inhibits the further dephosphorylation of the Ins4P thus formed: by 51% with 4 mM-LiCl and by 74% with 20 mM-LiCl.

**DISCUSSION**

Our results show that *Dictyostelium discoideum* homogenates possess phosphatases which can rapidly dephosphorylate Ins(1,4,5)P₃. The two presumptive dephosphorylation pathways can be summarized by Scheme 1.

![Scheme 1](image_url)

**Abbreviation**: 2,3-BG, 2,3-bisphosphoglycerate.

Although at first sight the phosphatase activities appear to be very different from those in higher organisms, on closer inspection many similarities can be found. *Dictyostelium* contains an Ins(1,4,5)P₃ 5-phosphatase activity which is Mg²⁺-dependent, sensitive to 2,3-bisphosphoglycerate, insensitive to Li⁺ and present in both particulate and soluble cell fractions. This is similar to the 5-phosphatase from rat liver and brain (Erneux et al., 1986; Shears et al., 1987), which also contains both soluble and particulate 5-phosphatase activities. Further dephosphorylation of Ins(1,4)P₂ by the *Dictyostelium* soluble cell fraction is also similar to the rat or bovine brain systems; Ins(1,4)P₂ is dephosphorylated in both systems by a 1-phosphatase to form Ins4P, which is then dephosphorylated to Ins (Inhorn et al., 1987; Delvaux et al., 1987b; Ragan et al., 1988). Furthermore, the Ins(1,4)P₂ 1-phosphatase and the Ins4P phosphatases of both systems are sensitive to Li⁺, with half-maximal inhibition at about 2.5 mM-LiCl, and are 10–100 times less sensitive to 2,3-bisphosphoglycerate than are their Ins(1,4,5)P₃ 5-phosphatase activities (Delvaux et al., 1987a,b). The *Dictyostelium* 5-phosphatase enzyme differs from these systems in that Ca²⁺ can replace Mg²⁺. In platelets and erythrocytes Ca²⁺ cannot replace Mg²⁺, and Ca²⁺ inhibits the Mg²⁺-activated activity with high affinity ($K_i = 70 \mu M$) (Downes et al., 1982; Connolly et al., 1985). We have used Ca²⁺ as a tool to elucidate the dephosphorylation pathway of Ins(1,4,5)P₃ in *Dictyostelium*, but have no indication for its physiological importance.

The major difference between Ins(1,4,5)P₃ dephosphorylation in *Dictyostelium* and that of higher organisms is, however, that in *Dictyostelium* most of the Ins(1,4,5)P₃ phosphatase activity is due to a predominantly soluble 1-phosphatase, which is highly Li⁺-sensitive [10-fold more sensitive than Ins(1,4)P₂ and 1-Ins1P 1-phosphatase activities]. This shows that, although many of the *Dictyostelium* Ins(1,4,5)P₃-dephosphorylating enzymes could be very similar to those of higher organisms, the metabolic pathway of Ins(1,4,5)P₃ dephosphorylation is very different, and a new InsP₂ isomer, Ins(4,5)P₂, is formed.

It is now important to investigate which InsP₂ is present *in vivo*. Nothing is known about the identity of any of the isomers of the inositol phosphates that are present *in vivo* in *Dictyostelium*, but this can be investigated by h.p.l.c. The high sensitivity of Ins(1,4,5)P₃ dephosphorylation for Li⁺ *in vitro* makes it interesting to see whether the dramatic effects that Li⁺ has on the determination of cell differentiation and pattern formation *in vivo* (Maeda, 1970; Sakai, 1973; Van Lookeren Campagne et al., 1988) are mediated through interference with the metabolism of the inositol (poly)phosphates.

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