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

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*Abbreviations used: Ins, myo-inositol; Ins1P, d-myo-inositol 1-phosphate; Ins2P, l-myo-inositol 1-phosphate; Ins3P, d-myo-inositol 4-phosphate; Ins4P, d-myo-inositol 4-phosphate; Ins1,4P2, d-myo-inositol 1,4-bisphosphate; Ins4,5P2, d-myo-inositol 4,5-bisphosphate; Ins1,4,5P3, d-myo-inositol 1,4,5-trisphosphate; InsP, InsP2, and InsP3, d-myo-inositol phosphates without specification of the phosphate position(s).† Present address: Department of Anatomy and Cell Biology, College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York, NY 10032, U.S.A.

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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 t-Ins1P (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 potentially 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 a more complicated phosphatase system. 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*.

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INTRODUCTION

The pivotal role of Ins(1,4,5)P3 as the second messenger for receptor-mediated Ca**²⁺** mobilization has been firmly established in a 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; Ackerman 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 (Hallcher & 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 Ca**²⁺** release from non-mitochondrial Ca**²⁺** 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 InsP3 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 Mn**²⁺**-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 phospho...
phosphorylates diacylglycerol to phosphatidic acid (Jimenez et al., 1988). Phosphatidylinositol 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)P$_3$. Here we report that Ins(1,4,5)P$_3$ 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)P$_2$ or Ins(4,5)P$_2$, which are both dephosphorylated, through Ins4P, to Ins.

**MATERIALS AND METHODS**

**Materials**

[2-$^3$H]Ins(1,4,5)P$_3$ (1.0 Ci/mmol) and l-[U-$^{14}$C]Ins1P (55 mCi/mmol) were from Amersham International. [2-$^3$H]Ins(1,4)P$_2$ (2.0 Ci/mmol), [4,5-$^{32}$P]Ins(1,4,5)P$_3$ (130 Ci/mmol), [2-$^3$H]Ins1P (5.4 Ci/mmol) and [2-$^3$H]-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 NH$_2$; 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 10$^7$ cells/cm$^2$ 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 × 10$^8$ 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-$^3$H]Ins(1,4,5)P$_3$, [4,5-$^{32}$P]Ins(1,4,5)P$_3$, [2-$^3$H]Ins(1,4)P$_2$, [2-$^3$H]Ins(4,5)P$_2$, [4,5-$^{32}$P]Ins(4,5)P$_2$ and/or l-[U-$^{14}$C]Ins1P was assayed in buffer A, in the presence of either 5 mM-MgCl$_2$ or 2.5 mM-CaCl$_2$, 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 radiolabelled 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-Na$_2$B$_4$O$_7$ (InsP and P$_2$); (3) 10 ml of 300 mM-ammonium formate/100 mM-formic acid (InsP$_2$); and (4) 10 ml of 750 mM-ammonium formate/100 mM-formic acid (InsP$_3$). 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 P$_2$, reaction products (usually with the internal standard of l-[U-$^{14}$C]Ins1P) were separated by anion-exchange h.p.l.c. as described in the Figure legends. Ins(1,4,5)P$_3$ 5-phosphatase activity from human erythrocyte 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)P$_2$**

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-phenylmethylsulphonyl fluoride, soya-bean trypsin inhibitor (50 μg/ml) and 5 mM-benzamidine]. The high-speed supernatant from 3 × 10$^8$ 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)P$_3$ 5-phosphatase activity binds to the column, whereas the Ins(1,4,5)P$_3$ 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 $^{32}$P- and $^3$H-labelled Ins(4,5)P$_2$ was prepared in an incubation (100 μl) containing 50 nCi of [4,5-$^{32}$P]Ins(1,4,5)P$_3$, 100 nCi of [2-$^3$H]Ins(1,4,5)P$_3$, 5 mM-MgCl$_2$, 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-tributylammonium 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-tributylammonium phosphate/4.5 mM-tributylammonium 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. [2-$^3$H]Ins(4,5)P$_2$ was prepared in parallel from 100 nCi of [2-$^3$H]Ins(1,4,5)P$_3$. 1988
Dephosphorylation of inositol trisphosphate in Dictyostelium

Table 1. Relative Ins(1,4,5)P3 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-MgCl₂</td>
<td>100</td>
<td>21</td>
<td>82</td>
</tr>
<tr>
<td>2.5 mM-CaCl₂</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-MgCl₂</td>
<td>21</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>2.5 mM-CaCl₂</td>
<td>11</td>
<td>10</td>
<td>1</td>
</tr>
</tbody>
</table>

RESULTS

General properties of Ins(1,4,5)P3 dephosphorylation in Dictyostelium

Ins(1,4,5)P3 can be rapidly dephosphorylated by a D. discoideum homogenate to InsP2. Activity is optimal at pH 7.0 and 5 mM-MgCl₂ (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)P3 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).

CaCl₂ (2.5 mM) can replace MgCl₂ 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)P3 phosphatase

The studies with the Ins(1,4,5)P3 phosphatase inhibitors Li⁺ and 2,3-bisphosphoglycerate suggest that in 5 mM-Mg⁺² Ins(1,4,5)P3 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 [4,5-³²P]Ins(1,4,5)P3 and [2-³²H]Ins(1,4,5)P3. The distribution of ³²P label between the 4- and 5-phosphates of InsP2 phosphatase activity was assayed in the soluble fraction from 5x10⁷ cells/ml with 0.5 nCi of [4,5-³²P]-Ins(1,4,5)P3 and 2.5 nCi of [2-³²H]Ins(1,4,5)P3 under the conditions indicated and described in the Materials and methods section. Incubations were terminated after 10 min when assayed in MgCl₂ or after 20 min when assayed in CaCl₂ and chromatographed on Dowex columns. The ratio between the ³²P and ³²H radioactivity in the InsP2 fractions was determined by liquid-scintillation counting, with a dual-label program. The ³²P/³²H ratios in InsP2 relative to that in Ins(1,4,5)P3 are given in parentheses. The incubation with human erythrocyte membranes, which contains only 5-phosphatase, revealed that the ³²P label was distributed over the 4- and 5-positions in the ratio 12:88. A low ³²P/³²H ratio in InsP2 therefore indicates 5-phosphatase activity, whereas a high ratio indicates 1- and/or 4-phosphatase activity.

Table 2. Ratio between ³²P and ³²H radioactivity in InsP2 produced by hydrolysis of a mixture of [4,5-³²P]Ins(1,4,5)P3 and [2-³²H]Ins(1,4,5)P3

<table>
<thead>
<tr>
<th>Activity (%)</th>
<th>³²P/³²H ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay with:</td>
<td></td>
</tr>
<tr>
<td>No addition</td>
<td>0.74 (1.00)</td>
</tr>
<tr>
<td>25 mM-LiCl</td>
<td>0.61 (0.82)</td>
</tr>
<tr>
<td>0.25 mM-2,3-bisphosphoglycerate</td>
<td>0.12 (0.16)</td>
</tr>
<tr>
<td>No additions</td>
<td>0.68 (0.92)</td>
</tr>
<tr>
<td>25 mM-LiCl</td>
<td>0.11 (0.15)</td>
</tr>
<tr>
<td>0.25 mM-2,3-bisphosphoglycerate</td>
<td>0.09 (0.12)</td>
</tr>
</tbody>
</table>

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by a 5-phosphatase, which is sensitive to 2,3-bisphosphoglycerate. Ca$^{2+}$ can only replace Mg$^{2+}$ for the 5-phosphatase. In the particulate fraction the 5-phosphatase and 1- and/or 4-phosphatase are about equally active.

Analysis of $^{32}$P/$^{3}$H ratios does not give a clear-cut answer about whether the Li$^{+}$-sensitive Ins(1,4,5)$P_3$ phosphatase activity dephosphorylates Ins(1,4,5)$P_3$ at the 1- or at the 4-position, as the fraction of $^{32}$P label in the 4-position is too small for accurate analysis. As we do know that the Ins$P_1$ thus formed still contains the 5-phosphate, the two possible isomers of this Ins$P_2$ are Ins(4,5)$P_2$ or Ins(1,5)$P_2$, and it is therefore termed Ins(x,5)$P_2$.

Dephosphorylation of Ins$P_2$

The two distinct pathways for the first step in Ins(1,4,5)$P_3$ dephosphorylation, measured in 5 mM-MgCl$_2$, have been shown above to yield Ins(x,5)$P_2$ (80%) and Ins(1,4)$P_2$ (20%). We used [2-$^3$H]Ins(1,4)$P_2$ 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)$P_2$ with the *Dictyostelium* soluble cell fraction shows that the Ins$P$ formed from Ins(1,4)$P_2$ is exclusively Ins4$P$ (Fig. 1).

To study the dephosphorylation of Ins(x,5)$P_2$ to Ins$P$, we synthesized and purified a $^{32}$P/$^{3}$H-labelled mixture of Ins(x,5)$P_2$ from [4,5-$^{32}$P]Ins(1,4,5)$P_3$ and [2-$^3$H]Ins(1,4,5)$P_3$ with a partially purified phosphatase.

A mixture of [4,5-$^{32}$P]Ins(1,4,5)$P_3$ and $[^3]$H]Ins(1,4,5)$P_3$ was incubated with a partially purified phosphatase from *Dictyostelium* as described in the Materials and methods section. The reaction mixture was chromatographed on a LiChrosorb reversed-phase column, and 0.6 ml fractions were collected; the radioactivity in 6 μl was determined with a dual-label program. The three fractions eluted around 12 min were combined. The $^{32}$P/$^{3}$H ratio in the substrate Ins(1,4,5)$P_3$ was 0.60, and that in the product Ins(x,5)$P_2$ was 0.61. The elution of commercial standards is indicated by the arrows; Ins(4,5)$P_2$ is eluted later than 15 min. O, $^{32}$P radioactivity; ●, $^3$H radioactivity.
Dephosphorylation
Inhibition
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3.
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activity acting
Ins(1,4,5)P3
inhibits
at
occurs
about 80
Ins(I,4,5)P3.
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1987;
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1985)
were
sensitive
of about 80
(see Fig. 2) was incubated with Dictyostelium soluble cell fraction for 60 min in the presence of 5 mM-MgCl2. 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 32P/3H ratio in the InsP peak is low (0.05, relative to 0.61 in substrate), indicating that it does not contain a 5-phosphogroup. Co-elution with authentic Ins4P and absence of a 5-phosphate in the InsP identifies the product as Ins4P, and the Ins(x,5)P2 substrate as Ins(4,5)P2. *32P radioactivity; •, 3H radioactivity.

Inhibition of Ins(1,4,5)P3 phosphatase by Li+

As shown in Table 1, the inhibitor-sensitivity of Ins(1,4,5)P3 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)P3 (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)P2, Ins4P and Ins1P (Hallencher & Sherman, 1980; Inhorn & Majerus, 1987; Gee et al., 1988). As shown in Fig. 4, Li+ effectively inhibits Ins(1,4,5)P3 phosphatase activity to a maximum of about 80%, if measured in 5 mM-Mg++ with 0.25 μM-Ins(1,4,5)P3. Half-maximal inhibition of this activity occurs at about 0.25 mM-LiCl. When phosphatase activity was measured in Ca++, Li+ had no effect (Fig. 4).

![Fig. 4. Effect of Li+ on Ins(1,4,5)P3 phosphatase activity in the soluble cell fraction of Dictyostelium homogenates](image)

Dephosphorylation of [2-3H]Ins(1,4,5)P3 (5 nCi; 0.25 μM) was assayed in the presence of different LiCl concentrations as described in the Materials and methods section, with either 5 mM-MgCl2 (●) or 2.5 mM-CaCl2 (○). The phosphatase activity data are expressed as percentages of the control without LiCl.

Inhibition of Ins(1,4,5)P3 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)P2 and Ins1P phosphatases (Delvaux et al., 1987a). In the Dictyostelium soluble cell fraction 2,3-bisphosphoglycerate inhibits the Ins(1,4,5)P3 phosphatase activity biphasically, when measured with 0.25 mM-Ins(1,4,5)P3 in 5 mM-Mg++ (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++, however, all the activity can be inhibited with high sensitivity, half-maximal inhibition occurring at about 3 μM (Fig. 5).

The combination of the effects of Li+ and 2,3-bisphosphoglycerate on the Dictyostelium Ins(1,4,5)P3 phosphatase activity (assuming that the inhibitor-sensitivities of the Dictyostelium and rat brain enzymes are similar) supplements the evidence presented above that in Mg++ Ins(1,4,5)P3 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++ apparently can only replace Mg++ in that part of the activity which is sensitive to 2,3-bisphosphoglycerate, so in Ca++ all activity is apparently due to a 5-phosphatase which has some similarities to the 5-phosphatase from higher organisms.

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Fig. 5. Effect of 2,3-bisphosphoglycerate on Ins(1,4,5)P$_3$ phosphatase activity in the soluble cell fraction of Dictyostelium homogenates

Dephosphorylation of [2-$^3$H]Ins(1,4,5)P$_3$ (5 nCi; 0.25 mM) 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.

Sensitivity of InsP$_i$ 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-$^3$H]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-$^3$H]Ins(4,5)P$_2$ from [2-$^3$H]Ins(1,4,5)P$_3$ (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-$^3$H]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 l-Ins1P dephosphorylation

myo-Inositol monophosphates can be derived from two different sources: (1) l-Ins1P formed from isomerization of D-glucose 6-phosphate catalysed by l-myo-inositol 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-myo-inositol 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$_3$] and does not discriminate between the two enantiomeric conformations of Ins1P (Eisenberg, 1967; Hallcher & Sherman, 1980; Ackermann et al., 1987). Furthermore, Li$^+$ is a potent inhibitor of InsP phosphatase activities (Naccarato et al., 1974; Hallcher & Sherman, 1980; Sherman et al., 1984; Ackermann et al., 1987; Delvaux et al., 1987b; Gee et al., 1988).
In Dictyostelium homogenates, t-Ins1P and Ins4P are dephosphorylated under conditions similar to those described for the above systems. Li⁺ inhibits the dephosphorylation of L-[U-¹⁴C]Ins1P in the soluble fraction of these homogenates, with half-maximal inhibition at about 2.5 mM (Fig 7). Furthermore, when [²⁻H]Ins(1,4)P₃ 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)

**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 Mg⁺²-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 t-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 Ins₄P isomerase, Ins₄(5,5)P₂, is formed.

It is now important to investigate which Ins₄P 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.

**REFERENCES**


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