Dephosphorylation of 1D-*myo*-inositol 1,4-bisphosphate in rat liver

Andrew J. MORRIS,*† Diane J. STOREY,* C. Peter DOWNES† and Robert H. MICHELL*§

*Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, U.K.,

and †Department of Cellular Pharmacology, Smith Kline & French Research Ltd., The Frythe, Welwyn, Herts. AL6 9AR, U.K.

Dephosphorylation of 1D-*myo*-inositol 1,4-bisphosphate [$\text{Ins}(1,4)\text{P}_2$] in rat liver is catalysed by a cytosolic phosphatase that removes the 1-phosphate group. The K_m for $\text{Ins}(1,4)\text{P}_2$ is approx. $17\ \mu\text{M}$. Li^+ (100 mM) causes 50 % inhibition of $\text{Ins}(1,4)\text{P}_2$ phosphatase activity when activity is measured at the very low substrate concentration of 10 nM, but on raising the substrate concentration to 100 μM there is a greater than 10-fold increase in sensitivity to Li^+ , suggesting that Li^+ acts mainly, but not entirely, as an uncompetitive inhibitor of $\text{Ins}(1,4)\text{P}_2$ phosphatase. In addition, rat liver cytosol shows Li^+ -sensitive phosphatase activity against 1D-*myo*-inositol 1-, 3- and 4-monophosphates. The $\text{Ins}(1,4)\text{P}_2$ 1-phosphatase and inositol monophosphatase activities all share an apparent M_r of 47×10^3 , as determined by gel-filtration chromatography. However, the $\text{Ins}(1,4)\text{P}_2$ 1-phosphatase is more sensitive to inactivation by heat, and can be separated from inositol monophosphatase activity by anion-exchange chromatography. We conclude that rat liver cytosol contains an $\text{Ins}(1,4)\text{P}_2$ 1-phosphatase that is distinct from, but in many ways similar to, inositol monophosphatase.

INTRODUCTION

$\text{Ins}(1,4,5)\text{P}_3$ is a second messenger that links cell-surface receptor activation to the release of intracellularly stored Ca^{2+} (Berridge & Irvine, 1984; Downes & Michell, 1985). A major cellular mechanism for inactivation of $\text{Ins}(1,4,5)\text{P}_3$ is dephosphorylation at the 5-position (Downes *et al.*, 1982; Storey *et al.*, 1984; Connolly *et al.*, 1985). Cells also contain further enzymes for the complete dephosphorylation of the $\text{Ins}(1,4)\text{P}_2$ derived from $\text{Ins}(1,4,5)\text{P}_3$ (Storey *et al.*, 1984; Hansen *et al.*, 1986; Shears *et al.*, 1987). The most extensively characterized of these enzymes is inositol monophosphatase, which dephosphorylates several isomers of *myo*-inositol monophosphate and is inhibited by Li^+ (Hallcher & Sherman, 1980; Takimoto *et al.*, 1985; Ackerman *et al.*, 1987; Gee *et al.*, 1988). However, when cells are preloaded with Li^+ and then stimulated through receptors that activate $\text{Ins}(1,4,5)\text{P}_3$ formation, Li^+ amplifies not only the accumulation of inositol monophosphate but also that of $\text{Ins}(1,4)\text{P}_2$ and $\text{Ins}(1,3,4)\text{P}_3$; the last compound is a metabolite of $\text{Ins}(1,4,5)\text{P}_3$ (Berridge *et al.*, 1982; Burgess *et al.*, 1985). Thus Li^+ must have effects on enzymes of inositol phosphate metabolism other than inositol monophosphatase.

We have previously reported results which suggested that: (a) rat liver cytosol contains separate phosphatase activities directed against the 1- and 4-phosphate groups of $\text{Ins}(1,4)\text{P}_2$, with only the 4-phosphatase being sensitive to inhibition by Li^+ ; (b) the $\text{Ins}(1,4)\text{P}_2$ 4-phosphatase and $\text{Ins}1\text{P}$ phosphatase activities of liver cytosol might be catalysed by the same enzyme (Storey *et al.*, 1984; Michell *et al.*, 1986). Ackerman *et al.* (1987) then reported that a partially purified inositol monophosphatase preparation from brain was also capable of

hydrolysing $\text{Ins}(1,4)\text{P}_2$. Very recently, however, inositol monophosphatase and $\text{Ins}(1,4)\text{P}_2$ phosphatase have been separately purified from brain (Takimoto *et al.*, 1987; Inhorn *et al.*, 1987; Inhorn & Majerus, 1987; Ragan *et al.*, 1988), but these studies have themselves disagreed on which of the phosphate groups of $\text{Ins}(1,4)\text{P}_2$ is attacked by the purified $\text{Ins}(1,4)\text{P}_2$ phosphatase.

In this paper, we briefly describe rat liver $\text{Ins}(1,4)\text{P}_2$ phosphatase and give evidence that it is different from the inositol monophosphatases of liver.

METHODS

Preparation of inositol phosphates

$[\text{32P}]\text{Ins}1\text{P}$ was prepared from $[\text{32P}]\text{PtdIns}$ that had been isolated from rat hepatocytes which were labelled with $[\text{32P}]\text{P}_i$ (25–50 $\mu\text{Ci}/\text{ml}$) in the presence of 230 nM-vasopressin under the conditions described by Palmer *et al.* (1986). PtdIns was isolated from a total lipid extract of the cells by ethanol precipitation (Lapetina & Michell, 1973) and was degraded to produce $\text{Ins}1\text{P}$ by either phosphoinositidase C-catalysed hydrolysis or by deacylation followed by gentle periodate oxidation and reduction (see below). For the first of these strategies, the lipid was emulsified in water. The source of phosphoinositidase C was a rat liver cytosolic fraction. This was prepared as described below and dialysed extensively against 2 mM-maleate/2 mM-Tris, pH 5.5. The cytosol was incubated with the lipid emulsion at 37 °C in the presence of 1 mM- Ca^{2+} , and the incubation was terminated by the addition of solvents to the final proportions chloroform/methanol/water (10:10:9, by vol.). The upper phase was taken, methanol removed under vacuum and the sample concentrated by freeze-

Abbreviations used: PtdIns , phosphatidylinositol; $\text{PtdIns}4\text{P}$, phosphatidylinositol 4-phosphate; InsP , InsP_2 , InsP_3 and InsP_4 , *myo*-inositol mono-, bis-, tris- and tetrakis-phosphates, with locants designated with reference to 1D-*myo*-inositol where appropriate.

† Present address: Department of Pharmacology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514, U.S.A.

§ To whom correspondence should be addressed.

drying. [^{32}P]Ins1P was separated by high-voltage paper electrophoresis from the [^{32}P]Ins(1:2 cyclic)P that was also produced by phosphoinositidase C-catalysed cleavage of PtdIns (Dawson & Clarke, 1972).

For the preparation of [^{32}P]Ins4P and Ins(1,[^{32}P]4) P_2 the starting point was PtdIns[^{32}P]4P isolated from human erythrocytes labelled with [^{32}P]P_i basically as described in King *et al.* (1987). An entire erythrocyte lipid extract was deacylated by transacylation to methylamine (Clarke & Dawson, 1981). For the preparation of Ins(1,[^{32}P]4) P_2 , the water-soluble ^{32}P -labelled products obtained were subjected to mild periodate oxidation (Brown *et al.*, 1959). In order to avoid attack on the inositol ring, incubation was for 20 min in the dark at room temperature, with an approx. 10-fold molar excess of 10 mM-sodium periodate. The incubation was quenched with a large excess of ethylene glycol. The glycolaldehyde phosphate derivatives thus formed were reduced with 1,1-dimethylhydrazine (Brown & Stewart, 1966), and Ins(1,[^{32}P]4) P_2 was separated from [^{32}P]P_i and Ins(1,[^{32}P]4,[^{32}P]5) P_3 also produced by anion-exchange chromatography on Bio-Rad AG1X8 (200–400 mesh, formate form) with ammonium formate/formic acid eluents (Downes & Michell, 1982). For the preparation of [^{32}P]Ins4P, glycerophosphoinositol 4-[^{32}P]phosphate was purified by anion-exchange chromatography of the water-soluble deacylation products of the total lipid extract and subjected to alkaline hydrolysis (Brockerhoff & Ballou, 1962). The glycerophosphoinositol 4-[^{32}P]phosphate was dissolved in 1 M-KOH and heated at 100 °C for 30 min in a sealed tube. The hydrolysate was cooled on ice, neutralized with 11 M-HClO₄, and the resultant precipitate removed before purification of Ins4P by anion-exchange chromatography as described above. The salt used to elute Ins4P and Ins(1,4) P_2 from anion-exchange columns was removed from the preparations of these compounds by the method of Hawkins *et al.* (1986). [^3H]Ins1P and [^3H]Ins(1,4) P_2 were purchased from New England Nuclear U.S.A. [^{14}C]Ins3P ($\equiv$ L-Ins1P) was purchased from Amersham International. Unlabelled D/L-Ins(1,4) P_2 was generously given by Dr. C. I. Ragan of Merck, Sharp and Dohme Research Laboratories.

Preparation of rat liver homogenates and subcellular fractions

Male Wistar rats were starved overnight, a lethal dose of sodium pentobarbitone in saline was administered, and the livers were cleared by perfusion with 250 mM-sucrose/50 mM-Tris, pH 7.2, containing 50 μM -phenylmethanesulphonyl fluoride. Excised livers were homogenized in the same medium; the homogenate was filtered through a 300 μm mesh and adjusted to a final dilution of 0.33 g/ml fresh wt. Cytosolic and mixed particulate fractions were prepared by centrifugation of the homogenate at 180 000 *g* for 1 h. The mixed particulate fraction was washed once by resuspension in homogenizing buffer, followed by repeated centrifugation. Protein concentrations were determined by the method of Bradford (1976).

Assay of inositol phosphate phosphatases

The dephosphorylation of ^{32}P -labelled inositol phosphates was monitored by measuring the release of [^{32}P]P_i by the method of Martin & Doty (1949), toluene being substituted for benzene. In the studies of the

dephosphorylation of ^3H - and ^{14}C -labelled inositol phosphates, anion-exchange chromatography on Bio-Rad AG1X8 (200–400 mesh, formate form) with ammonium formate/formic acid eluents was used to separate Ins from InsP and InsP₂ (Downes & Michell, 1982). The incubation buffer used contained 100 mM-KCl, 10 mM-NaCl, 10 mM-Hepes, pH 7.2, 1 mM-EGTA and 3 mM-MgSO₄. Incubations were at 37 °C. For most of the experiments described, inositol phosphates were included in the incubations at concentrations of 10–50 nM. In some cases the Ins(1,4) P_2 concentration was increased by the addition of D/L-Ins(1,4) P_2 .

RESULTS

Subcellular distribution of Ins1P, Ins3P, Ins4P and Ins(1,4) P_2 phosphatases in rat liver

A rat liver homogenate, and the cytosolic and total particulate fractions derived from it by differential centrifugation, were incubated with Ins1P, Ins3P, Ins4P and Ins(1,4) P_2 (at concentrations of 10–50 nM), and the rates of dephosphorylation of these inositol phosphates determined. Under these assay conditions, the kinetics of dephosphorylation of all of the inositol phosphates were first-order, because the concentrations of inositol phosphates employed were well below the K_m values of the relevant phosphatases (see below). The phosphatase activities were therefore quantified by determination of first-order rate constants, and these were then corrected for the dilution of the tissue from which the homogenates were derived (see Table 1). The phosphatase activities against all of the inositol phosphates studied were predominantly soluble. The three inositol monophosphates were hydrolysed at approximately equal rates under the assay conditions used, and Ins(1,4) P_2 was dephosphorylated much more rapidly.

Positional specificity of rat liver cytosolic Ins(1,4) P_2 phosphatase

[^3H]Ins(1,4) P_2 and Ins(1,[^{32}P]4) P_2 (at 10 and 50 nM respectively) were incubated with rat liver cytosol as described above. Incubations containing Ins(1,[^{32}P]4) P_2 were assayed for release of [^{32}P]P_i, and those containing

Table 1. Subcellular distribution of Ins1P, Ins3P, Ins4P and Ins(1,4) P_2 phosphatases in rat liver

Rat liver cytosol was incubated with the inositol phosphates shown at starting concentrations of 10–50 nM. Their rates of dephosphorylation were determined as explained in the text. The results shown are means $\pm$ S.E.M., corrected for dilution from the volume of starting tissue, for three to five independent determinations; ND, not determined.

Substrate	First-order rate constant (s ⁻¹)		
	Homogenate	Cytosolic fraction	Mixed particulate fraction
Ins1P	0.090 $\pm$ 0.007	0.081 $\pm$ 0.009	0.008 $\pm$ 0.002
Ins3P	ND	0.069 $\pm$ 0.001	ND
Ins4P	0.070 $\pm$ 0.011	0.071 $\pm$ 0.001	0.010 $\pm$ 0.001
Ins(1,4) P_2	0.48 $\pm$ 0.02	0.44 $\pm$ 0.04	0.07 $\pm$ 0.02

Fig. 1. Dephosphorylation of $[^3\text{H}]\text{Ins}(1,4)\text{P}_2$ and $\text{Ins}(1,[^{32}\text{P}]4)\text{P}_2$ by a rat liver homogenate

$[^3\text{H}]\text{Ins}(1,4)\text{P}_2$ (starting concn. 10 nM) and $\text{Ins}(1,[^{32}\text{P}]4)\text{P}_2$ (starting concn. 50 nM) were incubated with a freshly prepared rat liver homogenate under otherwise identical conditions as discussed in the text. The incubations were terminated, and those containing $\text{Ins}(1,[^{32}\text{P}]4)\text{P}_2$ were assayed for release of $[^{32}\text{P}]\text{P}_i$ ($\circ$), whereas the samples containing $[^3\text{H}]\text{Ins}(1,4)\text{P}_2$ were analysed by anion exchange chromatography on small resin columns to resolve inositol ($\bullet$) from InsP ($\blacktriangle$) and $\text{Ins}(1,4)\text{P}_2$ ($\blacksquare$). The data shown are means of duplicate determinations normalized to the total amount of ^3H or ^{32}P radioactivity in each incubation. This experiment has been repeated three times with similar results.

$[^3\text{H}]\text{Ins}(1,4)\text{P}_2$ were analysed by anion-exchange chromatography to separate Ins and InsP from $\text{Ins}(1,4)\text{P}_2$. The results are shown in Fig. 1. Dephosphorylation of $[^3\text{H}]\text{Ins}(1,4)\text{P}_2$ was rapid, with a concomitant accumulation of $[^3\text{H}]\text{InsP}$. This InsP was then dephosphorylated more slowly to yield $[^3\text{H}]\text{Ins}$. The kinetics of release of $[^{32}\text{P}]\text{P}_i$ from $\text{Ins}(1,[^{32}\text{P}]4)\text{P}_2$ followed the same time course as the accumulation of $[^3\text{H}]\text{Ins}$ rather than that of the initial dephosphorylation of $\text{Ins}(1,4)\text{P}_2$ to an InsP . This shows that the $\text{Ins}(1,4)\text{P}_2$ phosphatase activity of liver is directed primarily against the 1-phosphate group, giving $[^{32}\text{P}]\text{Ins}4\text{P}$ as its product, and that this is subsequently dephosphorylated and thus releases its ^{32}P as $[^{32}\text{P}]\text{P}_i$. The products of $[^3\text{H}]\text{Ins}(1,4)\text{P}_2$ dephosphorylation were further investigated by anion-exchange h.p.l.c., with an elution system that could resolve $\text{Ins}1\text{P}$ and $\text{Ins}4\text{P}$. $[^3\text{H}]\text{Ins}4\text{P}$ was the only product detected. When the concentration of $\text{Ins}(1,4)\text{P}_2$ was raised to 0.1 mM [i.e. approx. 6 times the K_m of $\text{Ins}(1,4)\text{P}_2$ phosphatase; see below] and the experiment repeated, $\text{Ins}4\text{P}$ was still the only labelled inositol monophosphate product (results not shown).

Comparison of the properties of rat liver $\text{Ins}(1,4)\text{P}_2$ phosphatase and inositol monophosphatase

We originally speculated that the same phosphatase might be responsible for dephosphorylation of $\text{Ins}1\text{P}$ and $\text{Ins}(1,4)\text{P}_2$ in rat liver homogenates (Storey *et al.*, 1984). Inositol monophosphatase is remarkably resistant to heat denaturation (Charalampous & Chen, 1966; Naccarato *et al.*, 1974). Inactivation by heating therefore provides a potential means of distinguishing inositol monophosphatase from other phosphatases that attack inositol phosphates. When rat liver cytosol was heated to 70 °C, there was a rapid denaturation of most of the protein, whereas inositol monophosphatase activity against either $\text{Ins}1\text{P}$ or $\text{Ins}4\text{P}$ remained undiminished

Fig. 2. Susceptibility to heat-inactivation of rat liver cytosolic $\text{Ins}1\text{P}$, $\text{Ins}4\text{P}$ and $\text{Ins}(1,4)\text{P}_2$ phosphatases

Rat liver cytosolic fraction was warmed to 70 °C as described in the text, then cooled on ice, and denatured protein was removed by centrifugation. The supernatants obtained were assayed for protein ($\blacksquare$) and for phosphatase activity against $\text{Ins}1\text{P}$ ($\blacktriangle$), $\text{Ins}4\text{P}$ ($\bullet$) and $\text{Ins}(1,4)\text{P}_2$ ($\circ$) by the methods described in the text. The data shown are means of duplicate determinations. The inositol phosphatase activities shown are normalized to those present in unheated cytosol. This experiment has been repeated once with similar results.

(Fig. 2). $\text{Ins}(1,4)\text{P}_2$ phosphatase activity did not start to decline until a substantial proportion of the total protein had been denatured, but was then quickly inactivated. Thus it appears that inositol monophosphatase and $\text{Ins}(1,4)\text{P}_2$ 1-phosphatase are separate enzymes, with the former very thermostable and the latter being thermally denatured relatively easily.

We also compared the chromatographic behaviours of inositol monophosphatases and $\text{Ins}(1,4)\text{P}_2$ 1-phosphatase. A 40–60 % -satd. $(\text{NH}_4)_2\text{SO}_4$ fraction of rat liver cytosol, which was enriched with respect to both $\text{Ins}1\text{P}$ phosphatase and $\text{Ins}(1,4)\text{P}_2$ phosphatase, was subjected to gel-filtration fast protein liquid chromatography (f.p.l.c.) on a calibrated Superose-12 column. $\text{Ins}1\text{P}$ phosphatase and $\text{Ins}(1,4)\text{P}_2$ 1-phosphatase were eluted in the same position, indicating that these activities share an approximate M_r of 47×10^3 (results not shown). The 40–60 % -satd. $(\text{NH}_4)_2\text{SO}_4$ fraction was also examined by anion-exchange fast protein liquid chromatography on a Mono-Q HR 10/10 column: the eluate was assayed for phosphatase activity against $\text{Ins}1\text{P}$ and $\text{Ins}(1,4)\text{P}_2$. Each phosphatase ran as a single peak of activity, with a recovery exceeding 80 % of the activity applied to the column, and the two activities were clearly separated (Fig. 3).

Inhibition of $\text{Ins}(1,4)\text{P}_2$ phosphatase by Li^+

Li^+ inhibits both inositol monophosphatase and the brain $\text{Ins}(1,4)\text{P}_2$ 1-phosphatase in an uncompetitive manner (Hallcher & Sherman, 1980; Takimoto *et al.*, 1985; Inhorn *et al.*, 1987; Gee *et al.*, 1988). The rat liver cytosolic $\text{Ins}(1,4)\text{P}_2$ phosphatase is also inhibited by Li^+ (Storey *et al.*, 1984). When $\text{Ins}(1,4)\text{P}_2$ 1-phosphatase activity was assayed with high and low substrate concentrations, in the presence of various concentrations of Li^+ , inhibition at any single Li^+ concentration was greater at the higher substrate concentrations (Fig. 3).

Fig. 3. Separation of rat liver cytosol Ins(1,4) P_2 and Ins1P phosphatases by anion-exchange fast protein liquid chromatography

A 1 ml portion of a dialysed 40–60% satd.-(NH₄)₂SO₄ fraction of rat liver cytosol was applied to a Mono-Q HR 10/10 anion-exchange fast-protein-liquid-chromatography column. The column was eluted at 1 ml/min with a buffer containing 50 mM-Tris, pH 7.2, 1 mM-EGTA, 1 mM-NaN₃ and a linear gradient of 0–500 mM-KCl (—) with a final wash of 1 M-KCl as shown. Fractions (1 ml) of the eluate were collected and assayed for phosphatase activity against Ins1P (●) and Ins(1,4) P_2 (○). The data shown are means of duplicate determinations of these activities. A similar separation has been seen in one other experiment.

This observation of increased inhibition when an increased proportion of the molecules in an enzyme population are complexed with substrate is a diagnostic indication that uncompetitive inhibition is occurring, probably as a component of a mixed mechanism that also includes an element of non-competitive inhibition (see below) (Cornish-Bowden, 1986).

The K_m of Ins(1,4) P_2 1-phosphatase for Ins(1,4) P_2 {assayed radiochemically using [³H]Ins(1,4) P_2 diluted with unlabelled D/L-Ins(1,4) P_2 } was $17.8 \pm 3.3 \mu\text{M}$ (mean $\pm$ S.E.M. for three determinations). With an Ins(1,4) P_2 concentration of 100 μM (i.e. approx. $6 \times K_m$), half-maximal inhibition occurred at approx. 10 mM-Li⁺, suggesting an approximate K_i for Li⁺ of 8 mM (Fig. 4). When the Ins(1,4) P_2 concentration was decreased to 10 nM (i.e. approx. $0.0006 \times K_m$), 100 mM-Li⁺ was needed to achieve half-maximal inhibition (Fig. 4). If Li⁺ was acting solely as an uncompetitive inhibitor, then the values of K_m and K_i determined above would predict that only a 4% inhibition of the enzyme should be observed in assays with 10 nM-Ins(1,4) P_2 as substrate. These results suggest that Li⁺ acts as both an uncompetitive and a non-competitive inhibitor of Ins(1,4) P_2 phosphatase, with binding of the inhibitor to the enzyme-substrate complex approx. 10 times stronger than to the free enzyme.

This conclusion is the same as that reached for bovine brain inositol monophosphatase as a result of a more detailed kinetic analysis by Ragan *et al.* (1988). In agreement with their study, we have found that the rat liver cytosolic Ins1P phosphatase shows an approximate K_m of 100 μM . Dephosphorylation of Ins1P, Ins3P and Ins4P by this phosphatase was inhibited by Li⁺, with half-maximal inhibition occurring at a Li⁺ concentration of approx. 10 mM when the substrate concentrations were 10–50 nM (results not shown).

DISCUSSION

The results reported above establish the major route of degradation of Ins(1,4) P_2 in rat liver homogenate or cytosol as dephosphorylation by a Li⁺-sensitive phosphatase that removes the 1-phosphate, a result that is different from that which we reported previously (Storey *et al.*, 1984; Michell *et al.*, 1986), but in accord with the conclusions reached by most other workers who have studied the dephosphorylation of Ins(1,4) P_2 by cell-free preparations from brain or liver (Moyer *et al.*, 1987; Inhorn *et al.*, 1987; Ackerman *et al.*, 1987; Ragan *et al.*, 1988). Our previous incorrect conclusion arose from: (a) the presence, in preparations of Ins(1,[³²P]4) P_2 made by activating the phosphoinositidase C of human erythrocyte plasma membranes with Ca²⁺, of a ³²P-labelled and phosphatase-sensitive contaminant; and (b) our resulting failure to recognize that the rapid liberation of [³²P]P_i from Ins(1,[³²P]4) P_2 is a two-step process in which [³²P]Ins4P is an intermediate. Despite the agreement between our new results and those of most other laboratories, there is still an unresolved conflict with the work of Takimoto *et al.* (1987). They have reported the isolation from brain of an Ins(1,4) P_2 phosphatase that is similar in general characteristics to those reported by other groups and ourselves, but which appears to be specific for the 4-phosphate of Ins(1,4) P_2 rather than the 1-phosphate. This apparent disagreement must await either verification or disproof from further studies.

In 1986, we briefly reported that liver Ins(1,4) P_2 phosphatase was more heat-sensitive than inositol monophosphatase (Michell *et al.*, 1986), and Inhorn *et al.* (1987) have since made a similar observation on the brain enzyme. At the same time, we noted that rat liver cytosolic Ins1P phosphatase was separable from Ins-

Fig. 4. Inhibition of rat liver cytosolic Ins(1,4) P_2 phosphatase by Li^+ at two different substrate concentrations

Rat liver cytosol was incubated with [^3H]Ins(1,4) P_2 at a starting concentration of either 10 nM (○) or 100 μM (●) [achieved by addition of D/L-Ins(1,4) P_2] and the rate of dephosphorylation of the Ins(1,4) P_2 determined at concentrations of Li^+ in the range 1 μM –0.1 M. The data shown are means of duplicate determinations normalized to enzyme activity determined in the absence of Li^+ . Ins(1,4) P_2 phosphatase activity was also determined in the presence of 0.1 M-NaCl at 100 μM -Ins(1,4) P_2 (■). This experiment is representative of three which gave similar results.

(1,4) P_2 1-phosphatase by anion-exchange chromatography, a result which is illustrated for liver in Fig. 3 and which has also been reported by Moyer *et al.* (1987), Inhorn & Majerus (1987) and Gee *et al.* (1988).

In summary, there now appears to be agreement that all inositol monophosphates are degraded by the same heat-stable phosphatase, and that a second phosphatase of similar M_r removes the 1-phosphate from Ins(1,4) P_2 [and probably also from Ins(1,3,4) P_3 (Shears *et al.*, 1987; Inhorn *et al.*, 1987)]. Both of these enzymes are subject to inhibition by Li^+ by a largely uncompetitive mechanism, with the monophosphatase the more sensitive. Given the rarity of this mechanism of inhibition and the other similarities between the two enzymes, it seems very likely that they are closely related proteins. Moreover, the fact that these two Li^+ -sensitive enzymes are responsible for two sequential reactions in the same pathway of degradation of inositol polyphosphates would appear to strengthen the case for these enzymes as major targets of Li^+ both in the relief of manic-depressive illness (Berridge *et al.*, 1982) and in the inositol-reversible teratogenic effect on *Xenopus* embryos (Busa, 1988).

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