Purification and properties of inositol-1,4-bisphosphatase from bovine brain

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Inositol-1,4-bisphosphatase has been purified 13000-fold from bovine brain supernatant. The enzyme is monomeric, with an apparent subunit M_r of 40000. Maximal hydrolytic rates were observed in Tris buffer, pH 7.8, in the presence of 9 mM-Mg²⁺. The enzyme acted as a 1-phosphatase, hydrolysing both inositol 1,4-bisphosphate [Ins(1,4)P₂] (K_m 0.04 mM) and inositol 1,3,4-trisphosphate [Ins(1,3,4)P₃] (K_m 0.5 mM) to inositol 4-phosphate and inositol 3,4-bisphosphate respectively. Li⁺ inhibited the hydrolysis of both substrates in an uncompetitive manner, with apparent K_i values of 9.63 mM and 0.46 mM for Ins(1,4)P₂ and Ins(1,3,4)P₃ respectively.

INTRODUCTION

Cleavage of PtdIns(4,5)P₂ by phospholipase C generates two second messengers, diacylglycerol and Ins(1,4,5)P₃ (for reviews see [1–4]). The latter molecule mobilizes the limited intracellular Ca²⁺ stores from a non-mitochondrial compartment, probably in the endoplasmic reticulum [5,6], and is rapidly metabolized to Ins(1,4)P₂ by membrane-bound and cytosolic 5-phosphatases [7]. Alternatively, Ins(1,4,5)P₃ undergoes a rapid phosphorylation and then dephosphorylation reaction to yield Ins(1,3,4)P₃ [8–11] via Ins(1,3,4,5)P₄ by the concerted action of 3-kinase and 5-phosphatase activities. Ins(1,3,4,5)P₄ has been reported to mediate entry of extracellular Ca²⁺ across the sea-urchin egg plasma membrane, thus prolonging the elevated concentrations of Ca²⁺ seen after receptor stimulation [12]. Ins(1,3,4)P₃ is metabolized by a cytosolic enzyme to Ins(3,4)P₂ [7,13], although Ins(1,3)P₂ has been reported to be a quantitatively minor product [14]. Regardless of how Ins(3,4)P₂ and Ins(1,4)P₂ are further dephosphorylated, their monophosphate products are all substrates for the same Li⁺-sensitive enzyme, inositol monophosphatase (EC 3.1.3.25), which has been purified and characterized in some detail [15,16]. The free inositol generated is then utilized for the resynthesis of PtdIns and polyphosphoinositides.

Until recently, Ins(1,4)P₂ was widely thought to be degraded to inositol via D-Ins(1)P, since this is the most abundant monophosphate found in the cerebral cortex of Li⁺-treated animals [17,18] and in thrombin-stimulated platelets [19]. However, D-Ins(4)P was shown to be the major product of Ins(1,4)P₂ hydrolysis in agonist-stimulated rat adrenal glomerulosa [20] and pituitary cells [21], implicating some other metabolite, possibly PtdIns, as the major source of D-Ins(1)P. In addition, we have recently shown [22] that brain or liver homogenates/supernatants (rat or cow) contain 1-phosphatases which catalyse the hydrolysis of Ins(1,4)P₂ almost exclusively to Ins(4)P. Using an enzyme preparation partially purified

from brain supernatant by ion-exchange chromatography, Inhorn *et al.* [13] demonstrated that Ins(1,4)P₂ and Ins(1,3,4)P₃ were both hydrolysed by 1-phosphatase enzymes to Ins(4)P and Ins(3,4)P₂, respectively, the latter compound being further degraded by a 4-phosphatase to D-Ins(3)P [L-Ins(1)P].

In the present paper we report the purification to homogeneity of bovine brain Ins(1,4)P₂-1-phosphatase. Its molecular and enzymic properties are described, and we show that it catalyses the hydrolysis of both Ins(1,4)P₂ and Ins(1,3,4)P₃.

MATERIALS AND METHODS

Materials

Frozen bovine brains were obtained from Imperial Laboratories, Salisbury, Wilts., U.K. DL-Ins(1,3,4,5)P₄ and Ins(1,3)P₂ were prepared as described by Billington & Baker [23], DL-Ins(3,4)P₂ and DL-Ins(1,4)P₂ as described by Angyal & Tate [24], and inositol monophosphates as described by Billington *et al.* [25]. [Ins-2-³H(n)]-Ins(1,3,4,5)P₄ (5 Ci/mmol) was obtained from New England Nuclear (Du Pont, Stevenage, Herts., U.K.). Other materials were obtained from sources as noted previously [16,22].

Assays

Ins(1,4)P₂ phosphatase and inositol monophosphatase were assayed either by colorimetric determination of released P_i [26] or by radiochemical assays [22]. Ins-(1,3)P₂-, Ins(3,4)P₂-, Ins(1,4,5)P₃-, Ins(1,3,4)P₃- and Ins(1,3,4,5)P₄-phosphatases were assayed by colorimetric determination of released P_i.

Preparation of [³H]Ins(1,3,4)P₃

[³H]Ins(1,3,4)P₃ was prepared from [³H]Ins(1,3,4,5)P₄ by enzymic hydrolysis using rat liver plasma membranes. The reaction mixture contained 4.25 ml of buffer (20 mM-Hepes, 110 mM-KCl, 10 mM-NaCl, 1 mM-K₂HPO₄, 9 mM-MgCl₂, 10 mM-levamisole, pH 7.2), 500 μl of [³H]-

Abbreviations used: PtdIns(4,5)P₂, phosphatidylinositol 4,5-bisphosphate; Ins(1)P, *myo*-inositol 1-phosphate [similarly Ins(4)P]; Ins(1,4,5)P₃, *myo*-inositol 1,4-bisphosphate [similarly Ins(1,3)P₂, Ins(3,4)P₂, Ins(1,3,4)P₃, Ins(1,4,5)P₃, Ins(1,3,4,5)P₄].

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Table 1. Purification of inositol-1,4-bisphosphatase

Purification was as described in the Materials and methods section. Activity was measured by the radiochemical assay; 1 unit of activity hydrolyses 1 μ mol of substrate/min at 37 °C.

Fraction	Total activity (m-units)	Total protein (mg)	Specific activity (m-units/mg)	Yield (%)
Supernatant	91 700	19 800	4.6	100
(NH ₄) ₂ SO ₄ ppt.	74 300	2970	25.0	81
Q-Sepharose	33 300	90	370	36
Sepacryl S-200	16 400	5.8	2830	18
Phenyl-Superose	4640	0.19	24 400	5.1
Mono Q	1170	0.019	61 300	1.3

Ins(1,3,4,5) P_4 (5 μ Ci) and 250 μ l of plasma-membrane preparation, giving > 90 % conversion of [3 H]Ins P_4 in 30 min at 37 °C (levamisole is an alkaline phosphatase inhibitor [27]). The reaction was terminated by heating in boiling water for 3 min. The sample was diluted to 25 ml with water and applied to a 1 ml Dowex (formate form) column equilibrated with water. Elution was performed with a 0–1.2 M-ammonium formate/0.1 M-formic acid gradient (total volume 180 ml) at 1 ml/min; 2 ml fractions were collected and counted for 3 H radioactivity in order to determine the position of the Ins P_3 peak. Peak fractions were pooled, freeze-dried and desalted on Sephadex G-10 (see below). After freeze-drying, the residue was dissolved in 3 ml of water. Purity was > 98 % when assessed by h.p.l.c. (see below).

Hydrolysis of [3 H]Ins(1,3,4) P_3

Reaction mixtures (0.3 ml) contained 30 000 d.p.m. of [3 H]Ins(1,3,4) P_3 (9 nM; 5 Ci/mmol) in 50 mM-Tris/HCl/250 mM-KCl/9 mM-MgCl₂, pH 7.8, and sufficient purified enzyme for > 70 % conversion into [3 H]Ins P_2 . After 60 min at 37 °C, the reaction was terminated by heating in boiling water for 3 min. The sample was diluted to 2 ml with 7 % (v/v) propan-1-ol and desalted on a Sephadex G-10 column (1.0 cm internal diam. $\times$ 30 cm) equilibrated with 7 % propan-1-ol [28]. The flow rate was 1 ml/min, and 1 ml fractions were collected. Fractions containing 3 H radioactivity were pooled and rotary-evaporated under reduced pressure. The residue was taken up in 100 μ l of water and analysed by h.p.l.c. (see below). Hydrolyses using [3 H]Ins(1,4) P_2 (5 Ci/mmol) were performed exactly as described above.

H.p.l.c. analysis of inositol phosphates

Samples (80 μ l) were subjected to h.p.l.c. on a Spherisorb SAX column (250 mm $\times$ 4.6 mm internal diam.; 5 μ m particle size). For separation of [3 H]Ins(1) P and [3 H]Ins(4) P the mobile phase was 44 mM-ammonium acetate, pH 3.7 (adjusted with H₃PO₄). For separation of inositol bisphosphates the mobile phase was 150 mM-ammonium acetate, pH 3.7 (adjusted with H₃PO₄). Under these conditions Ins(1,3) P_2 and Ins(1,4) P_2 were co-eluted, but both were baseline-separated from Ins(3,4) P_2 .

Purification of Ins(1,4) P_2 phosphate

Unless otherwise indicated, all operations were carried out at 0–4 °C. Two bovine brains were used as the starting material. Brain supernatant was prepared and then fractionated with solid (NH₄)₂SO₄ as previously

described [16]. Proteins precipitated between 40 and 65 % saturation were resuspended in approx. 200 ml of 50 mM-Tris/HCl, pH 8.0, and dialysed overnight against 2.5 l of the same buffer. Any insoluble material was removed by centrifugation at 30 000 *g* for 30 min.

Q-Sepharose chromatography

Dialysed material was applied at 5 ml/min to a Q-Sepharose (Pharmacia, Milton Keynes, Bucks., U.K.) column (2.5 cm internal diam. $\times$ 40 cm) equilibrated with 50 mM-Tris/HCl buffer, pH 8.0. The column was washed with 100 ml of 50 mM-Tris/HCl buffer, pH 8.0, and developed with a linear NaCl gradient (0–0.3 M) in a total volume of 1.5 litres of this buffer. Fractions (10 ml) were assayed for Ins(1,4) P_2 -phosphatase activity.

Sepacryl S-200 chromatography

Active fractions from Q-Sepharose were pooled and concentrated to 5 ml by ultrafiltration through an Amicon PM10 membrane. The concentrated sample was chromatographed on a Sepacryl S-200 column (2.5 cm internal diam. $\times$ 80 cm) equilibrated with 50 mM-Tris/HCl (pH 8.0)/0.2 M-NaCl/0.1 mM-EGTA/0.1 mM-EDTA at 1.5 ml/min. Active fractions were pooled, concentrated as above to 5 ml, and adjusted to 33 % saturation with (NH₄)₂SO₄ by addition of 2.5 ml of a saturated solution in 20 mM-Tris/HCl, pH 7.0 (at room temperature).

Phenyl-Superose chromatography

The protein was filtered through a 0.22 μ m-pore-size membrane and applied to an HR5/5 phenyl-Superose column (Pharmacia) equilibrated at room temperature with a 33 %-satd. solution of (NH₄)₂SO₄ in 20 mM-Tris/HCl, pH 7.0 (buffer A). Buffer B was 20 mM-Tris/HCl, pH 7.0. The column was washed with 5 ml of A + B (7:3, v/v) and developed with a linear gradient of A + B from 7:3 to 2:3 (v/v) (total vol. 70 ml, flow rate 1 ml/min). Active fractions were pooled, diluted to 50 ml with buffer B and concentrated to a final volume of 1.1 ml.

Mono Q chromatography

The protein was diluted to 10 ml with 50 mM-Tris/HCl, pH 7.0, and applied to an HR5/5 Mono Q column (Pharmacia) equilibrated with the same buffer. Proteins were eluted with a linear gradient of NaCl (0.09–0.2 M) in 50 mM-Tris/HCl buffer, pH 7.0 (total vol. 40 ml, flow rate 0.1 ml/min). Active fractions were pooled and stored frozen at –20 °C in 0.5 ml portions.

Fig. 1. SDS/polyacrylamide-gel electrophoresis of inositol-1,4-bisphosphatase

Purified enzyme (2 μ g) was analysed by electrophoresis as described in the Materials and methods section and detected by staining with Coomassie Blue. The M_r was determined by reference to markers of M_r values 67000, 45000, 29000, 17000, 12000.

Miscellaneous methods

Inositol monophosphatase was purified as described previously [16]. SDS/polyacrylamide-gel electrophoresis was performed in 12.5% polyacrylamide gels (0.75 mm $\times$ 8 cm $\times$ 7 cm) in the Laemmli [29] buffer system. Protein concentration was measured by the method of Bradford [30].

RESULTS

Purification of Ins(1,4) P_2 -phosphatase

The initial stages of the purification were similar to those used for the purification of inositol monophosphatase [16]. Ion-exchange conditions were modified so as to allow maximal separation of inositol monophosphatase and Ins(1,4) P_2 -phosphatase. Further purification by gel filtration on Sephacryl S-200, hydrophobic interaction on phenyl-Superose, and finally a second ion-exchange step on Mono Q, yielded an enzyme preparation free of inositol monophosphatase activity. Two batches of enzyme were prepared by this procedure. In the first, a 10000-fold purification was achieved (sp. activity 42000 munits/mg) in a yield of 8%. However, a minor contaminant of subunit M_r 30000 (not inositol monophosphatase) was present, and its final removal proved quite difficult. However, by discarding the leading and trailing edges of the activity

Fig. 2. Mg^{2+} dependence of inositol-1,4-bisphosphatase

Activity was measured by the radiochemical assay. The buffer used was 50 mM-Tris/HCl, pH 8.0, containing different amounts of $MgCl_2$ (0–20 mM). $\bullet$, Inositol monophosphatase; $\blacksquare$, inositol-1,4-bisphosphatase.

Fig. 3. pH optimum of inositol-1,4-bisphosphatase

Activity was measured by the radiochemical assay. Buffers (50 mM) were Mes/NaOH (pH 5.5, 6.0 and 6.5) and Tris/HCl (pH 7.0, 7.5, 8.0, 8.5, and 9.0). $MgCl_2$ was included (9 mM) in each case.

peaks in the second purification, a homogeneous preparation was obtained, but with lower yield. The progress of this purification from two bovine brains is shown in Table 1. The final enzyme preparation had a specific activity of 61 300 munits/mg, indicating a 13000-fold purification from brain supernatant.

Molecular properties

We previously reported the native M_r of bovine brain Ins(1,4) P_2 -phosphatase by gel filtration to be 46000 [16]. Analysis of the purified enzyme by SDS/polyacrylamide-gel electrophoresis revealed a single polypeptide species of subunit M_r 40000 (Fig. 1). These results are consistent with a monomeric structure.

Mg^{2+} -dependence

Ins(1,4) P_2 -phosphatase had an absolute requirement for Mg^{2+} and achieved a maximal rate in the presence of 9 mM- Mg^{2+} . In contrast, inositol monophosphatase activity was maximal with 2.3 mM- Mg^{2+} (Fig. 2).

pH optimum

The pH optimum of the enzyme was determined in either Mes or Tris buffers in the range pH 5.5–9.0. The optimum was found to be pH 7.8 (Fig. 3).

Fig. 4. Effect of KCl concentration on inositol-1,4-bisphosphatase

Activity was measured by the radiochemical assay. The buffer used was 50 mM-Tris/HCl containing different amounts of KCl (0–800 mM).

Table 2. Substrate specificity of inositol-1,4-bisphosphatase

Activities were determined either by colorimetric assay of P_i or by radiochemical assay. Rates were determined with 0.1 mM substrates and are expressed relative to the rate for DL-Ins(1,4) P_2 . The amount of enzyme used gave approx. 30% hydrolysis of DL-Ins(1,4) P_2 in 15 min; 10 times this amount of enzyme was used for the other putative substrates for the same period of time.

Substrate	Rate (%)
DL-Ins(1,4) P_2	100
DL-Ins(1,3,4) P_3	9.8
D-Ins(1,4,5) P_3	< 1
Ins(1,3) P_2	< 1
DL-Ins(3,4) P_2	< 1
DL-Ins(1) P	< 1
DL-Ins(1,3,4,5) P_4	< 1

Effect of KCl concentration on activity

The effect of varying the KCl concentration on enzyme activity is shown in Fig. 4. Activity was near maximal over a wide range of concentrations (0–300 mM), but was significantly inhibited at concentrations above 400 mM.

Substrate specificity

A number of inositol phosphates were examined in order to determine the specificity of the purified enzyme (Table 2). Ins(1,3) P_2 , DL-Ins(3,4) P_2 , DL-Ins(1,3,4,5) P_4 , D-Ins(1,4,5) P_3 and DL-Ins(1) P were not hydrolysed. However, DL-Ins(1,3,4) P_3 was hydrolysed at about one-tenth of the rate observed for DL-Ins(1,4) P_2 at 0.1 mM substrate. A similar ratio of hydrolytic rates was observed for the two substrates when crude brain supernatant was used as the source of enzyme (results not shown).

Product specificity

We have previously shown that crude brain supernatant hydrolyses [3H]Ins(1,4) P_2 , generating 98% [3H]Ins(4) P and 2% [3H]Ins(1) P [22]. Purified Ins(1,4) P_2 -phosphatase generated only [3H]Ins(4) P . In addition, we have examined the hydrolysis of [3H]Ins(1,3,4) P_3 by

Table 3. Kinetic constants for inositol-1,4-bisphosphatase

Hydrolysis of Ins(1,4) P_2 was measured by radiochemical assay using optimized buffer conditions (50 mM-Tris/HCl/250 mM-KCl/9 mM-MgCl₂, pH 8.0). Substrate concentrations (mM) were 1, 0.2, 0.1, 0.0333, 0.0167, 0.01. Assays were conducted in the presence of LiCl (0, 3.0, 10 or 30 mM). Hydrolysis of Ins(1,3,4) P_3 was measured by colorimetric determination of released P_i in the same buffer. Substrate concentrations (mM) were 2, 0.5, 0.20, 0.125, 0.1. LiCl concentrations were 0, 0.5 or 2 mM. Initial rates were fitted to a Michaelis–Menten expression by non-linear least-squares regression analysis.

Substrate	K_m (mM)	K_i (Li ⁺) (mM)	Relative V_{max}
DL-Ins(1,4) P_2	0.04	9.63	1
DL-Ins(1,3,4) P_3	0.50	0.48	1.03

pure enzyme and found [3H]Ins(3,4) P_2 to be the sole bisphosphate product.

Enantiomeric specificity

With the purified enzyme, the ratio of the hydrolytic rates for D-Ins(1,4) P_2 and L-Ins(1,4) P_2 was found to be 130:1. When a crude brain supernatant fraction was used, the ratio was decreased to 12.5:1.

Effect of Li⁺

Hydrolysis of both Ins(1,4) P_2 and Ins(1,3,4) P_3 by purified enzyme was inhibited by Li⁺ in an uncompetitive manner, with K_i values of 9.63 mM and 0.48 mM respectively. K_m values for these two substrates were 0.04 mM and 0.5 mM respectively (Table 3).

DISCUSSION

Ins(1,4,5) P_3 is metabolized in a complex series of phosphorylation and/or dephosphorylation reactions and ultimately returned to the free inositol pool. There have been conflicting reports on the identity of the metabolic intermediates produced, and hence on the specificity of the enzymes catalysing these reactions. Both Ins(1) P and Ins(4) P have been reported to be products of Ins(1,4) P_2 hydrolysis in rat brain and liver [18,31], although more recent work suggests that only trace quantities of Ins(1) P are produced [22]. In addition, Imai & Gershengorn [21] studied the kinetics of inositol phosphate formation in agonist-stimulated cells and concluded that Ins(1) P could not be produced from Ins(1,4) P_2 . Although it now seems clear that Ins(1,3,4) P_3 is hydrolysed predominantly to Ins(3,4) P_2 [32], the 1-phosphatase activity responsible has been variously attributed to Ins(1,4) P_2 -phosphatase [3], inositol monophosphatase [14] or a Li⁺-insensitive enzyme [7]. In the present study we therefore sought to purify Ins(1,4) P_2 -phosphatase to homogeneity, and hence to clarify its role in the degradation of inositol phosphates.

The purified enzyme hydrolysed both Ins(1,4) P_2 and Ins(1,3,4) P_3 , although at a substrate concentration of 0.1 mM the trisphosphate was hydrolysed at roughly one-tenth the rate. This difference in rate is largely explained

by the 12-fold difference in K_m values for the two substrates. These results suggest a much slower return of $\text{Ins}(1,4,5)\text{P}_3$ to free inositol via $\text{Ins}(1,3,4,5)\text{P}_4$ and $\text{Ins}(1,3,4)\text{P}_3$ than by the more direct dephosphorylation route via $\text{Ins}(1,4)\text{P}_2$.

Purified $\text{Ins}(1,4)\text{P}_2$ -phosphatase catalysed the hydrolysis of both $\text{Ins}(1,4)\text{P}_2$ and $\text{Ins}(1,3,4)\text{P}_3$, generating $\text{Ins}(4)\text{P}$ and $\text{Ins}(3,4)\text{P}_2$ respectively. These are the major products found after hydrolyses catalysed by crude supernatants [22,32], indicating little involvement of either 3- or 4-phosphatases in the degradation of $\text{Ins}(1,3,4)\text{P}_3$. That $\text{Ins}(1,4)\text{P}_2$ -phosphatase is the only 1-phosphatase enzyme involved is supported by the finding that relative rates of hydrolysis of $\text{Ins}(1,4)\text{P}_2$ and $\text{Ins}(1,3,4)\text{P}_3$ are similar for both crude supernatant and pure enzyme. Inhorn *et al.* [13] have suggested the name inositol polyphosphate 1-phosphatase for the enzyme that we have purified. However, since we have shown that several 1-phosphorylated inositol polyphosphates are not substrates, we suggest that the name $\text{Ins}(1,4)\text{P}_2/\text{Ins}(1,3,4)\text{P}_3$ 1-phosphatase is more appropriate.

The purified enzyme was shown to be enantiomer-specific, and only hydrolysed D- $\text{Ins}(1,4)\text{P}_2$ to any appreciable extent. However, crude bovine brain supernatant did contain an enzyme, probably a non-specific phosphatase, capable of hydrolysing the L-enantiomer, thus explaining the difference in enantiomeric specificity for supernatant and purified $\text{Ins}(1,4)\text{P}_2/\text{Ins}(1,3,4)\text{P}_3$ 1-phosphatase. Although the individual enantiomers of $\text{Ins}(1,3,4)\text{P}_3$ were not available to us, it is reasonable to assume that L- $\text{Ins}(1,3,4)\text{P}_3$ would not be a substrate for $\text{Ins}(1,4)\text{P}_2/\text{Ins}(1,3,4)\text{P}_3$ 1-phosphatase.

Moyer *et al.* [33] have partially purified a rat liver enzyme that hydrolyses $\text{Ins}(1,4)\text{P}_2$ to $\text{Ins}(4)\text{P}$. This enzyme preparation catalysed hydrolysis of $\text{Ins}(1,3,4)\text{P}_3$ (product not identified), the reaction being inhibited by $\text{Ins}(1,4)\text{P}_2$. These results are consistent with the notion that, as in bovine brain, a single enzyme is responsible for hydrolysis of both $\text{Ins}(1,4)\text{P}_2$ and $\text{Ins}(1,3,4)\text{P}_3$. Confirmation will require purification of the rat liver enzyme to homogeneity. The rat brain enzyme has recently been purified to homogeneity [34]. Despite some similarities to the bovine brain enzyme (M_r , Mg^{2+} -dependence, Li^+ -sensitivity), the rat enzyme acts as a 4-phosphatase, generating $\text{Ins}(1)\text{P}$ from $\text{Ins}(1,4)\text{P}_2$. This result is at variance with much of the literature and will require independent confirmation.

In agonist-stimulated cells treated with Li^+ , $\text{Ins}(1)\text{P}$, $\text{Ins}(4)\text{P}$, $\text{Ins}(1,4)\text{P}_2$ and $\text{Ins}(1,3,4)\text{P}_3$ accumulate [19,35,36]. Raised amounts of $\text{Ins}(1)\text{P}$ and $\text{Ins}(4)\text{P}$ are readily explained in terms of the specificity of inositol monophosphatase and its sensitivity to Li^+ (K_i values 1.0 mM and 0.28 mM respectively [16]). Since we have shown that Li^+ is an inhibitor of $\text{Ins}(1,4)\text{P}_2/\text{Ins}(1,3,4)\text{P}_3$ 1-phosphatase, the increase in both $\text{Ins}(1,4)\text{P}_2$ and $\text{Ins}(1,3,4)\text{P}_3$ may result from direct action of Li^+ on this enzyme. However, although a low K_i is observed for $\text{Ins}(1,3,4)\text{P}_3$ hydrolysis, the high K_m for this substrate means that little enzyme- $\text{Ins}(1,3,4)\text{P}_3$ complex, on which Li^+ can act, will be available *in vivo* unless the substrate concentration is high. On the other hand, $\text{Ins}(1,3,4)\text{P}_3$ hydrolysis may be prevented by $\text{Ins}(1,4)\text{P}_2$ since the latter substrate has a much lower K_m and will compete effectively for the active site of $\text{Ins}(1,4)\text{P}_2/\text{Ins}(1,3,4)\text{P}_3$ 1-phosphatase. The situation is further complicated in

that the degree of Li^+ inhibition of the various steps may also vary with time after receptor stimulation as metabolite concentrations fluctuate. Although the kinetic parameters of $\text{Ins}(1,4)\text{P}_2/\text{Ins}(1,3,4)\text{P}_3$ 1-phosphatase are now known, the determination of metabolite concentrations *in vivo*, in the presence of different concentrations of Li^+ , is essential if the complex effects of Li^+ on metabolic pathways controlled by the enzyme are to be fully understood. Although the concentrations of many inositol phosphates are influenced by Li^+ it may exert its effects by inhibiting just two enzymes, inositol monophosphatase and $\text{Ins}(1,4)\text{P}_2/\text{Ins}(1,3,4)\text{P}_3$ 1-phosphatase.

Finally, the purification factor obtained for homogeneous $\text{Ins}(1,4)\text{P}_2/\text{Ins}(1,3,4)\text{P}_3$ 1-phosphatase indicates that the enzyme represents less than 0.01% of the protein present in bovine brain supernatant, compared with 0.1% for inositol monophosphatase. Although the enzyme was monomeric (M_r 46000), and clearly different structurally from inositol monophosphatase, their absolute requirement for Mg^{2+} , similar pH optima and identical mode of inhibition by Li^+ , coupled with the obvious similarity in their respective substrates, suggests that their active sites and catalytic mechanisms may be closely related.

REFERENCES

1. Nishizuka, Y. (1986) *Science* **233**, 305–312
2. Majerus, P. W., Connolly, T. M., Deckmyn, H., Ross, T. S., Bross, T. E., Ishii, H., Bansal, V. S. & Wilson, D. B. (1986) *Science* **234**, 1519–1526
3. Berridge, M. J. (1984) *Biochem. J.* **220**, 345–360
4. Abdel-Latif, A. A., (1986) *Pharmacol. Rev.* **38**, 227–272
5. Streb, H., Irvine, R. F., Berridge, M. J. & Schulz, I. (1983) *Nature (London)* **306**, 67–69
6. Burgess, G. M., Godfrey, P. P., McKinney, J. S., Berridge, M. J., Irvine, R. F. & Putney, J. W. (1984) *Nature (London)* **309**, 63–66
7. Shears, S. B., Storey, D. J., Morris, A. J., Cubitt, A. B., Parry, J. B., Michell, R. H. & Kirk, C. J. (1987) *Biochem. J.* **242**, 393–402
8. Batty, J. R., Nahorski, S. R. & Irvine, R. F. (1985) *Biochem. J.* **232**, 211–215
9. Irvine, R. F., Letcher, A. J., Heslop, J. P. & Berridge, M. J. (1986) *Nature (London)* **320**, 631–634
10. Hawkins, P. T., Stephens, L. & Downes, C. P. (1986) *Biochem. J.* **238**, 507–516
11. Downes, C. P., Hawkins, P. T. & Irvine, R. F. (1986) *Biochem. J.* **238**, 501–506
12. Irvine, R. F. & Moor, R. M. (1986) *Biochem. J.* **240**, 917–920
13. Inhorn, R. C., Bansal, V. S. & Majerus, P. W. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2170–2174
14. Dillon, S. B., Murray, J. J. & Snyderman, R. (1987) *Biochem. Biophys. Res. Commun.* **144**, 264–270
15. Takimoto, K., Okada, M., Matsuda, Y. & Nakagawa, H. (1985) *J. Biochem. (Tokyo)* **98**, 363–370
16. Gee, N. S., Ragan, C. I., Watling, K. J., Aspley, S., Jackson, R. G. & Reid, G. G. (1988) *Biochem. J.* **249**, 883–889
17. Sherman, W. R., Munsell, L. Y., Gish, B. G. & Honchar, M. P. (1985) *J. Neurochem.* **44**, 798–807
18. Ackerman, K. E., Gish, B. G., Honchar, M. P. & Sherman, W. R. (1987) *Biochem. J.* **242**, 517–524
19. Siess, W. (1985) *FEBS Lett.* **185**, 151–156
20. Balla, T., Baukal, A. J., Guillemette, G., Morgan, R. O. & Catt, K. J. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9323–9327

21. Imai, A. & Gershengorn, M. C. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8540–8544
22. Ragan, C. I., Watling, K. J., Gee, N. S., Aspley, S., Jackson, R. G., Reid, G. G., Baker, R., Billington, D. C., Barnaby, R. J. & Leeson, P. D. (1988) *Biochem. J.* **249**, 143–148
23. Billington, D. C. & Baker, R. (1987) *J. Chem. Soc. Chem. Commun.* 1011–1013
24. Angyal, S. J. & Tate, M. E. (1961) *J. Chem. Soc.* 4122–4128
25. Billington, D. C., Baker, R., Kulagowski, J. J. & Mower, I. M. (1987) *J. Chem. Soc. Chem. Commun.* 314–316
26. Itaya, K. & Ui, M. (1966) *Clin. Chim. Acta* **14**, 361–366
27. van Belle, H. (1972) *Biochim. Biophys. Acta* **289**, 158–168
28. Wilson, D. B., Connolly, T. M., Bross, T. E., Majerus, P. W., Sherman, W. R., Tyler, A. N., Rubin, L. J. & Brown, J. E. (1985) *J. Biol. Chem.* **260**, 13496–13501
29. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
30. Bradford, M. (1976) *Anal. Biochem.* **72**, 248–252
31. Storey, D. J., Shears, S. B., Kirk, C. J. & Michell, R. H. (1984) *Nature (London)* **312**, 374–376
32. Shears, S. B., Parry, J. B., Tang, E. K. Y., Irvine, R. F., Michell, R. H. & Kirk, C. J. (1987) *Biochem. J.* **246**, 139–147
33. Moyer, J. D., Reizes, O., Dean, N. M. & Malinowski, N. (1987) *Biochem. Biophys. Res. Commun.* **146**, 1018–1026
34. Takimoto, K., Motoyama, N., Okada, M. & Nagakawa, H. (1987) *Biochim. Biophys. Acta* **929**, 327–335
35. Burgess, G. M., McKinney, J. S., Irvine, R. F. & Putney, J. W. (1985) *Biochem. J.* **232**, 237–243
36. Hansen, C. A., Mah, S. & Williamson, J. R. (1986) *J. Biol. Chem.* **261**, 8100–8103

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