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Purification and properties of *myo*-inositol-1-phosphatase from bovine brain

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myo-Inositol-1-phosphatase from bovine brain was purified over 2000-fold. The native enzyme has a M_r of 59000, and on SDS/polyacrylamide-gel electrophoresis the subunit M_r was 31000. Thus the native enzyme is a dimer of two apparently identical subunits. The enzyme, purified to a specific activity of more than 300 units/mg of protein (1 unit of enzyme activity corresponds to the release of 1 μmol of P_i/h at 37 °C), catalysed the hydrolysis of a variety of phosphorylated compounds, the best one, in terms of V/K_m, being D-*myo*-inositol 1-phosphate. Kinetic constants of compounds tested, including both isomers of glycerophosphate and two deoxy forms of β-glycerophosphate, were measured. They show the importance of the two hydroxyl groups which are adjacent to the phosphate in *myo*-inositol 1-phosphate. With a wide variety of substrates Li^+ was found to be an uncompetitive inhibitor whose K_i varied with substrate structure.

INTRODUCTION

The existence of *myo*-inositol-1-phosphatase (EC 3.1.3.25) has been known for more than 20 years (Chen & Charalampous, 1966; Eisenberg, 1967). The enzyme lies at an important point in the pathway of *myo*-inositol generation, since it catalyses the hydrolysis of both D- and L-enantiomers of *myo*-inositol 1-phosphate (Ins1*P*) (Eisenberg, 1967; Hallcher & Sherman, 1980). L-Ins1*P* is derived from glucose 6-phosphate via L-*myo*-insitol-1-phosphate synthase (Eisenberg, 1967; Mauck *et al.*, 1980), whereas D-Ins1*P* is derived from the hydrolysis of phosphatidylinositol phospholipids (Paulus & Kennedy, 1960). This second pathway is of great current interest, since the primary products of phosphatidylinositol 4,5-bisphosphate hydrolysis are inositol 1,4,5-trisphosphate and diacylglycerol, which have been shown to be intracellular second messengers. These molecules have been demonstrated to mediate the effects of a variety of hormones, neurotransmitters and growth factors (for reviews, see Berridge, 1986; Downes, 1986).

The other major reason for the interest in this enzyme is that it is inhibited by Li^+ (Naccarato *et al.*, 1974; Hallcher & Sherman, 1980; Takimoto *et al.*, 1985). This inhibition is interesting since it has been found that Li^+, which is widely used in the treatment of manic depression, causes a rise in the cortical concentration of Ins1*P* in rat brain on both acute (Allison *et al.*, 1976) and chronic (Sherman *et al.*, 1981) treatment.

Takimoto *et al.* (1985) have purified the rat brain *myo*-inositol-1-phosphatase and have reported some of its properties. Most of the work on the bovine enzyme reported to date has been performed on fairly crude enzyme preparations from bovine brain, with specific activities of about 45 units (1 unit corresponds to release of 1 μmol of P_i/h at 37 °C)/mg (Hallcher & Sherman, 1980; Ackerman *et al.*, 1987). Aspley *et al.* (1987) report having purified the enzyme to homogeneity, but do not give details of the preparation and say only that the rates of catalysis, K_m values and inhibition by Li^+ for the turnover of inositol monophosphates with this preparation are similar to those observed with crude preparations.

In the present paper we report the purification of *myo*-inositol-1-phosphatase by a factor of more than 2000 compared with the crude brain extract, and the examination of the physical properties of the enzyme. Structure–activity relationships for a variety of phosphorylated compounds were studied, and the inhibition by Li^+ with a variety of substrates was investigated.

MATERIALS AND METHODS

Enzyme source

Brains were obtained from freshly killed cattle at the local abattoir. They were transported to the laboratory in ice, and the two hemispheres were chopped into small pieces before being frozen in liquid N_2. The brains were then stored at −80 °C until used in the enzyme preparations.

Chemicals

D-*myo*-Inositol 1-phosphate (D-Ins1*P*) was obtained from Amersham (Paris, France). LiCl and $MgCl_2$ of the highest purity were purchased from Aldrich (Strasbourg, France), as was 4-nitrophenyl phosphate. All other compounds tested as substrates were obtained from Sigma, except isopropyl phosphate and propan-1-ol 2-phosphate, which were synthesized as described below. Bio-Gel HTP and Affi-Gel Blue were obtained from Bio-Rad, and Polybuffer 74 and PBE 94 gel were purchased from Pharmacia. M_r markers for SDS/polyacrylamide-gel electrophoresis were obtained from Bio-Rad, and

Abbreviation used: Ins1*P*, *myo*-inositol 1-phosphate; Ins2*P*, *myo*-inositol 2-phosphate.

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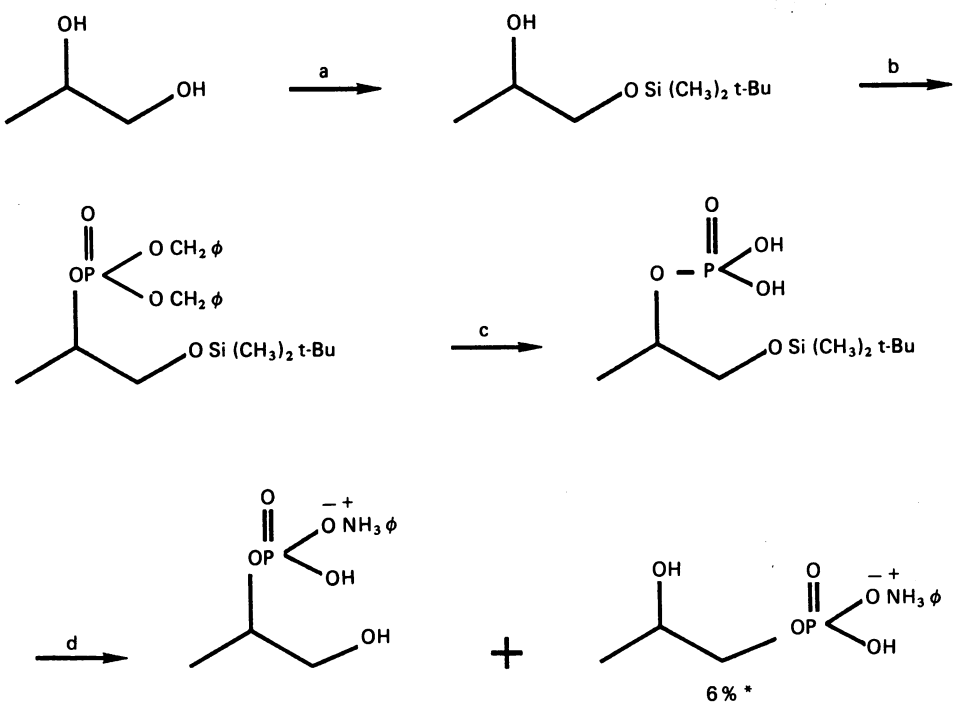


Scheme 1. Synthesis of propan-1-ol 2-phosphate

Steps: (a) t-Bu$(CH_3)_2$SiCl (1 equiv.), Et_3N (1.2 equiv.), dimethyl aminopyridine (catalytic amount), CH_2Cl_2.
(b) n-BuLi (1 equiv.), tetrahydrofuran, 0 °C; $(C_6H_5CH_2O)_2P(O)-O-P(O)(OCH_2C_6H_5)_2$ (1 equiv.), 0 °C 1.5 h (Khorana & Todd, 1953; Chouinard & Bartlett, 1986).
(c) H_2, Pd/C, C_2H_5OH, 1 atm. (101 kPa).
(d) Tetrahydrofuran/water (1:1, v/v), HCl (6 M) 1 drop, overnight room temperature; aniline (1 equiv.).
Symbol: ϕ, C_6H_5. * Yield evaluated by ^1H and ^{31}P n.m.r.

those used in h.p.l.c. gel filtration were obtained from Sigma or Boehringer Mannheim. All other materials were high-purity preparations from commercial sources.

Syntheses of isopropyl phosphate and propan-1-ol 2-phosphate

The synthesis of isopropyl phosphate, which was obtained as an anilinium salt, was performed as described by Zwierzak & Kluba (1971).

Propan-1-ol 2-phosphate was obtained as a mono-anilinium salt from propane-1,2-diol as described in reaction Scheme 1.

Assays

Enzyme activity was measured by determination of P_i liberated during the reaction, by using a slightly modified version of a method originally described by Meek & Nicoletti (1986). The molybdate reagent was prepared as follows: 80 ml of water + 5 ml of H_2SO_4 + 10 ml of 10% (v/v) Triton X-100, from which P_i had been removed by stirring with Dowex 1X-8 resin + 5 ml of 10% (w/v) ammonium molybdate. The enzymic reaction volume was usually 1 ml and, after the reaction had been started by the addition of enzyme, 200 μl samples were removed at different times and added to 800 μl of the molybdate reagent. This mixture was left for 20 min before the A_{350} was read. In order to preserve substrate, however, many of the experiments using D-Ins1*P* were performed in reaction volumes of 200 μl, with samples being removed for P_i determination. The molybdate reagent was calibrated by the addition of known amounts of P_i, and the change in A_{350} versus P_i was found to be linear in the range of 0–100 nmol of P_i, with about 76 nmol of P_i being required to give an A_{350} change of 1. In the cases where the phosphorylated substrates were labile in the molybdate reagent, P_i was assayed by the method of Black & Jones (1983). In assays with 4-nitrophenyl phosphate as a substrate, the reaction was monitored spectroscopically by following the release of the nitrophenolate ion at 400 nm by using ϵ_{400} at pH 8.0 of 17.3 $mM^{-1} \cdot cm^{-1}$. All assays were performed at 37 °C in buffer that usually comprised 0.05 M-Tris/HCl (pH 8.0) containing 0.1 mM-EGTA and 2 mM-$MgCl_2$. Assays on fractions collected from columns were usually performed with 4 mM-2′-AMP as substrate in the presence of 2 mM-$MgCl_2$. Other routine assays were performed under the same conditions with 1 mM-Ins1*P* as substrate.

Protein concentrations were determined by the method of Bradford (1976), with bovine serum albumin as standard.

Electrophoresis

SDS/polyacrylamide-gel electrophoresis was carried out by the method of Laemmli (1970). Proteins were detected by $AgNO_3$ staining, by using the fixation protocol of Irie *et al.* (1982) and the staining method of Morrissey (1981).

Gel-filtration chromatography

The M_r of the native enzyme was determined by using two (30 cm × 0.75 cm) Beckman Spherogel TSK-3000 SW

h.p.l.c. gel-filtration columns connected in series with a Spherogel TSK-SWPRE guard column. The protein was eluted at 0.5 ml/min with 0.1 M-Tris/HCl (pH 7.0) containing 0.1 M-KCl and 0.1 mM-EGTA. The eluted protein was detected at 220 nm with a Waters model 481 variable-wavelength detector, and 0.25 ml fractions of the eluate were collected. About 20 μg of the enzyme was concentrated to a volume of about 70 μl with a Centricon 30 micro-concentrator (Amicon) before injection on to the columns.

Data analysis

Kinetic data were analysed by a least-squares method with a program written by Duggleby (1981). Individual saturation curves were fitted to eqns. (1) and (2) to obtain values of V and V/K_m respectively:

$$v = \frac{V}{\left(1+\frac{K_m}{[S]}\right)} \quad (1)$$

$$v = \frac{\frac{V}{K_m}}{\left(\frac{1}{K_m}+\frac{1}{[S]}\right)} \quad (2)$$

The data obtained in the Li^+ inhibition experiments were fitted to eqn. (3), which is descriptive of uncompetitive inhibition and from which the estimates of K_i were derived:

$$v = \frac{V}{\left(1+\frac{K_m}{[S]}+\frac{[Li^+]}{K_i}\right)} \quad (3)$$

However, the sets of data at each $[Li^+]$ were also analysed individually, the data being inverted and fitted to the Lineweaver–Burk form of eqn. (1). In all forms of inhibition other than uncompetitive inhibition, the slopes of the Lineweaver–Burk plots vary linearly with inhibitor concentration [I] as described in eqn. (4):

$$\text{Slope} = \frac{K_m[I]}{K_i V}+\frac{K_m}{V} \quad (4)$$

where K_i is the dissociation constant of the EI complex. Thus the slopes obtained from the Lineweaver–Burk plots were fitted to eqn. (4) to see if they varied linearly with $[Li^+]$.

RESULTS

Enzyme purification

The purification method was based on that adopted by Takimoto *et al.* (1985) in their purification of rat brain *myo*-inositol-1-phosphatase. The chopped pieces corresponding to one bovine brain were thawed at room temperature before being homogenized in approx. 1 litre of cold 0.05 M-Tris/HCl (pH 8.0) containing 0.1 mM-EGTA, in a Polytron tissue grinder. The homogenate was then centrifuged at 14400 *g* for 30 min; this and all subsequent steps were performed at 4 °C. The supernatant was decanted and the enzyme was precipitated with a 40–60%-satd. solution of $(NH_4)_2SO_4$. After dialysis, the enzyme was loaded on to a DEAE-cellulose DE-52 column (~ 500 ml) and eluted with a 0–0.5 M-KCl gradient in 0.05 M-Tris/HCl (pH 8.0) containing 0.1 mM-EGTA. When 2′-AMP was used as substrate, a small peak of phosphatase activity was eluted just before the main peak. This was similar to that observed by Takimoto *et al.* (1985), although in our case this phosphatase activity formed a smaller proportion of the total activity. Motoyama *et al.* (1987) found this phosphatase activity formed a smaller proportion of the total activity. Motoyama *et al.* (1987) found this the enzyme pooled from this column.

The enzyme was further purified by passage through Sephadex G-150 and Bio-Gel HTP columns as described by Takimoto *et al.* (1985), except that the enzyme was eluted from the Bio-Gel HTP column with a 0–0.5 M-$(NH_4)_2SO_4$ gradient in 0.05 M-Tris/HCl (pH 8.0) containing 0.1 mM-EGTA. Pooled fractions from this column were then precipitated with 80%-satd. $(NH_4)_2SO_4$. After centrifugation at 21000 *g* for 30 min, the pellet was resuspended in 2 ml of 0.05 M-Tris/HCl (pH 8.0) containing 0.1 mM-EGTA, and was then dialysed against this buffer. For 2 h before loading on the chromatofocusing column, the enzyme was dialysed against

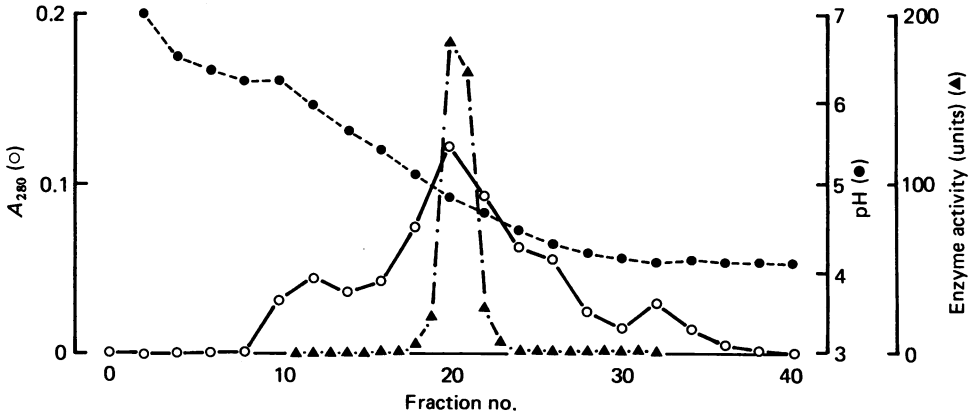


Fig. 1. Chromatofocusing column chromatography of pooled enzyme fractions from the Bio-Gel HTP column

The chromatofocusing column (13 cm × 0.5 cm) was equilibrated with 10 column vol. of 0.025 M-histidine/HCl (pH 6.2), followed by 2 column vol. of 1:8-diluted Polybuffer 74 (pH 4.0) before addition of the enzyme in a volume of 2.5 ml. The enzyme was then eluted with 7 column vol. of 1:8-diluted Polybuffer 74 (pH 4.0) at 24 ml/h, and 2 ml fractions were collected. All buffers were degassed before use.

Table 1. Purification of bovine brain *myo*-inositol-1-phosphatase

'Crude extract' is the supernatant of the brain homogenate obtained by centrifugation at 14400 *g* for 30 min. Activities were measured with 4 mM-2'-AMP as substrate.

	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Purification (fold)	Yield (%)
Crude extract	7800	4270	0.69	1	100
$(NH_4)_2SO_4$ fractionation	1400	2940	2.1	3	69
DE-52	120	1685	14	20	39
Sephadex G-150	16.6	882	53	77	21
Bio-Gel HTP	4.54	576	127	184	13
Chromatofocusing	0.46	350	771	1117	8
Affi-Gel Blue	0.17	256	1506	2183	6

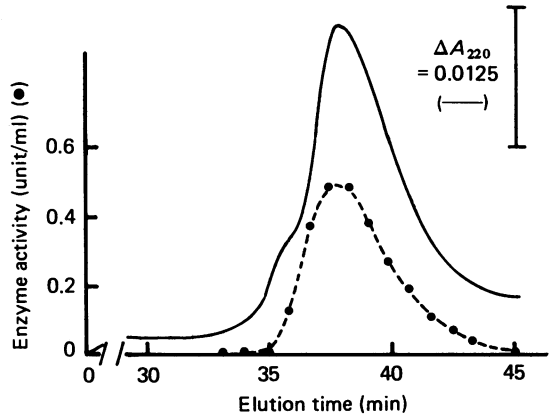


Fig. 2. Gel-filtration chromatography of *myo*-inositol-1-phosphatase on TSK-3000 SW columns

For details see the Materials and methods section. The M_r of the enzyme was estimated by comparison with the elution times of commercially purchased marker proteins chromatographed under identical conditions. These elution times were: ferritin (M_r 450000), 27.8 min; catalase (M_r 240000), 32.6 min; aldolase (M_r 158000), 33.0 min; bovine albumin (M_r 132000), 34.9 min; chicken albumin (M_r 45000), 37.8 min; lactalbumin (M_r 14500), 44.8 min. 44.8 min.

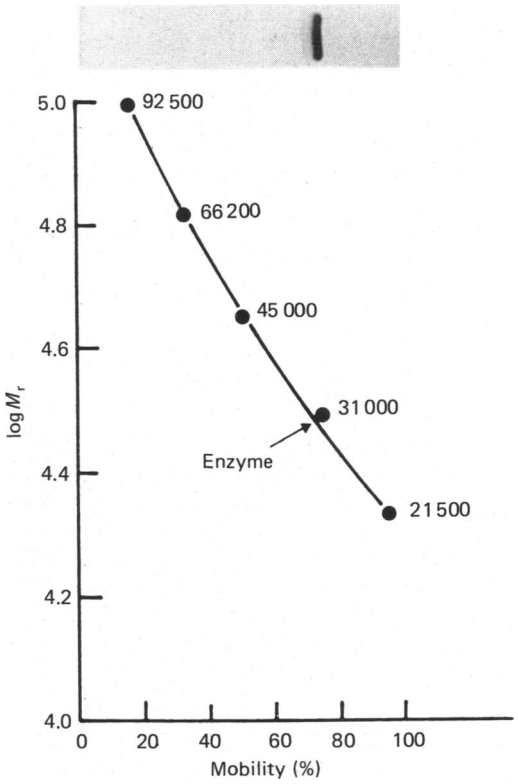


Fig. 3. SDS/polyacrylamide-gel electrophoresis and estimation of M_r of *myo*-inositol-1-phosphatase

Electrophoresis was done in a 12%-polyacrylamide gel. The M_r was estimated by comparison with the mobilities of commercially purchased marker proteins: phosphorylase *b* (M_r 92500), bovine serum albumin (M_r 66200), ovalbumin (M_r 45000), carbonic anhydrase (M_r 31000) and trypsin inhibitor (M_r 21500).

0.025 M-histidine/HCl (pH 6.2). The enzyme was then loaded on to the column and eluted with Polybuffer 74 adjusted to pH 4.0. The elution profile is shown in Fig. 1, which shows the enzyme to have a pI of about 4.8, which is similar to that reported by Takimoto *et al.* (1985) for the rat brain enzyme. The final stage of the preparation was to chromatograph the enzyme on an Affi-Gel Blue column as described by Hallcher & Sherman (1980).

The purification is summarized in Table 1. One difference between this preparation and that of the rat brain enzyme (Takimoto *et al.*, 1985) is that the specific activity of the purified bovine enzyme is higher than that of the rat enzyme. One explanation for this may be that the enzymes have different catalytic-centre activities.

Physical properties

Fig. 2 shows the elution profile of the enzyme on TSK-3000SW gel-filtration columns. The enzymic activity and the protein peak coincide. The M_r of the native enzyme was estimated to be 59000, which is very close to the 58800 observed by Hallcher & Sherman (1980). There is a small shoulder on the leading edge of the protein absorbance peak, which corresponds to a species of M_r between 90000 and 120000. This may represent the presence of a small amount of a contaminant protein or may be due to the presence of a small amount of tetrameric enzyme. However, only Naccarato *et al.*

Table 2. Substrate specificity of *myo*-inositol-1-phosphatase

Assays were performed under the standard conditions described in the Materials and methods section. When tested at 15 mM, the following compounds were found not to be substrates: phosphoenolpyruvate; *O*-phosphorylethanolamine; isopropyl phosphate; 5′-AMP; 2′,3′-cyclic AMP.

Compound	V (% of that for D-Ins1*P*)	V/K_m (% of that for D-Ins1*P*)	K_m (mM)
D-Ins1*P*	100	100	0.13
β-Glycerophosphate	118	45	0.38
2′-AMP	157	39	0.58
L-α-Glycerophosphate	22	4	0.87
Propan-1-ol 2-phosphate	8.4	0.07	15
4-Nitrophenyl phosphate	2.6	0.07	5

(1974) have reported higher-M_r species of the enzyme. They found that the enzyme from rat mammary gland appeared to exist as an M_r-210000 species which on heating was converted mainly into an M_r-52000 species. However in their gel-filtration chromatography elution profile, although most of the *myo*-inositol-1-phosphatase activity corresponded to an M_r -52000 species, there was a shoulder in the peak of enzymic activity which corresponded to a protein of M_r about 100000. Thus it is possible that multiple polymeric forms of the enzyme exist.

Fig. 3 shows that on SDS/polyacrylamide-gel electrophoresis the major protein band coincides with an M_r of 31000, indicating that the native form of the enzyme is a dimer comprising two apparently identical monomers. These M_r values are very similar to the 55000 and 29000 for the native enzyme and monomer found by Takimoto *et al.* (1985) for the rat brain enzyme.

As reported by both Hallcher & Sherman (1980) and Takimoto *et al.* (1985), the enzyme is resistant to heating, and our enzyme retained 66% of its activity after heating for 15 min at 70 °C.

Substrate specificity

As described previously by Hallcher & Sherman (1980) and Takimoto *et al.* (1985), the enzyme was inactive in the absence of Mg^{2+}. Using several different batches of the enzyme, we determined the initial-rate data for D-Ins1*P* as substrate. We found a K_m value of 0.125 ± 0.018 mM ($n=5$), which is similar to previous determinations (Hallcher & Sherman, 1980; Takimoto *et al.*, 1985; Aspley *et al.*, 1987). Included in this value of K_m are values for enzyme purified in the same way as described above, but where the pools from the columns were less stringently selected so as to decrease the loss of enzymic activity; this produced batches of enzyme with specific activities greater than 300 units/mg of protein. There was no apparent difference in K_m for D-Ins1*P* and 2′-AMP and the inhibition by Li^+ with these batches and with the enzyme purified to 1500 units/mg. Thus in the subsequent experiments enzyme purified to specific activities in the range 300–1500 units/mg was used.

A variety of phosphorylated compounds were tested as substrates, and the kinetic parameters determined in these experiments were compared with those obtained for D-Ins1*P* for the same enzyme. The results are shown in Table 2; the best substrate of those tested in terms of V/K_m was D-Ins1*P*. Although D-Ins1*P* has the lowest K_m, both β-glycerophosphate and 2′-AMP have somewhat higher V values, which may indicate that the catalytic step of phosphate hydrolysis of these substrates is enhanced. Table 2 shows that, unlike Hallcher & Sherman (1980) we found that both α- and β-glycerophosphates were substrates with β-glycerophosphate being more active. These substrate activities agree with the findings of Takimoto *et al.* (1985) for the rat brain enzyme.

In addition to those compounds shown in Table 2, inositol 2-phosphate (Ins2*P*) was tested as a substrate. Some difficulty arose here because we found that this compound supplied by Sigma contains 1.9% of P_i and, more importantly, 3.1% of Ins1*P* as determined by ^{31}P n.m.r. We tested this compound as a substrate over a range of concentrations of total labile phosphate (Ins1*P* + Ins2*P*, as measured by alkaline-phosphatase hydrolysis) of 0.1–1 mM. Expected velocities for the hydrolysis of the Ins1*P* contained in the assays were calculated by using the known values of V and K_m and compared with the observed velocities. At all concentrations, the observed velocities were greater than the velocities calculated for Ins1*P* hydrolysis. These calculated velocities ranged from 75% of the observed velocity at 1 mM total labile phosphate to 38% at 0.1 mM total labile phosphate. Thus the hydrolysis of the Ins1*P* contaminant does not account for all of the observed velocity. Thus it would seem that Ins2*P* is being hydrolysed. In addition, the calculations for the expected velocities for Ins1*P* hydrolysis do not take into account the report (Hallcher & Sherman, 1980), that Ins2*P* is a competitive inhibitor of the enzyme, with a K_i of 0.3 mM. If this is incorporated into the calculations of the expected rates of hydrolysis of Ins1*P*, the resultant calculated velocities are even lower percentages of the observed velocities.

Owing to the presence of the contaminant Ins1*P*, we were unable to calculate values of V, V/K_m and K_m, but the evidence does indicate that Ins2*P* is a substrate for *myo*-inositol-1-phosphatase. This is contrary to findings by both Eisenberg (1967) and Hallcher & Sherman (1980). Takimoto *et al.* (1985) did not report having tested Ins2*P* as a substrate for the purified rat brain enzyme; however, Chen & Charalampous (1966) reported Ins2*P* to be a substrate for the yeast enzyme.

In the series of glycerophosphate analogues, ranging from β-glycerophosphate to isopropyl phosphate, there was no substrate activity when there were no hydroxyl

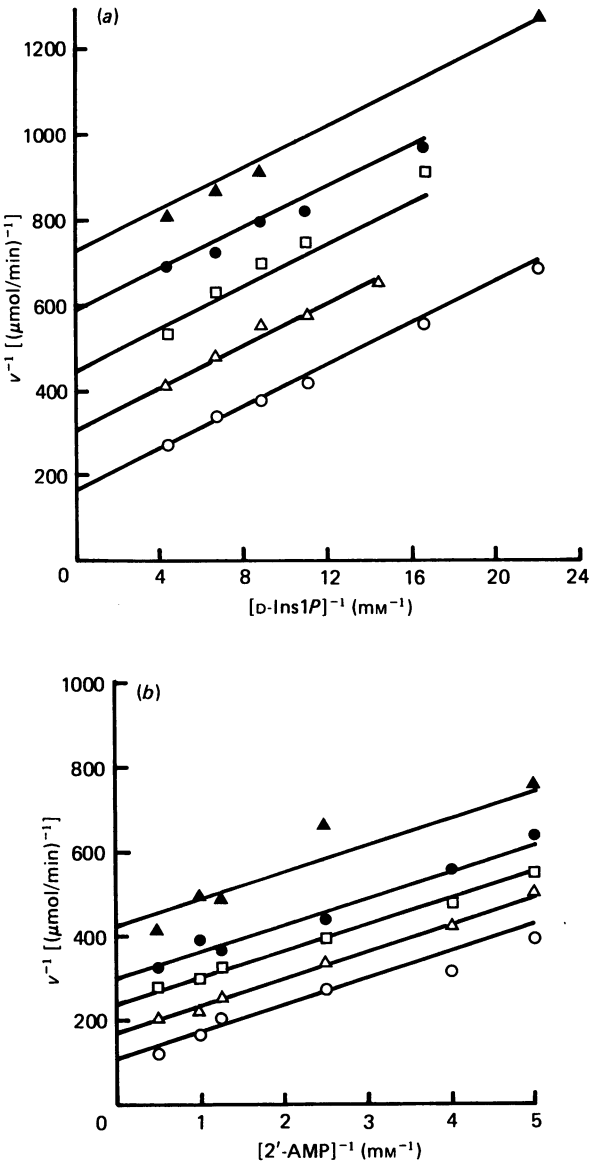


Fig. 4. Inhibition of enzyme activity by Li^+ with either D-Ins1*P* (*a*) or 2'-AMP (*b*) as substrate

Enzymic activities were measured as 37 °C in 0.05 M-Tris/HCl (pH 8.0) containing 0.1 mM-EGTA and 2 mM-$MgCl_2$, and the velocities were determined as μmol of P_i formed/min in the reaction mixture. With D-Ins1*P* (*a*) the concentrations of LiCl used were 0 mM (○), 0.5 mM (△), 1 mM (□), 1.5 mM (●) or 2 mM (▲). With 2'-AMP (*b*) the concentrations of LiCl used were 0 mM (○), 1 mM (△), 2 mM (□), 3 mM (●) or 5 mM (▲). The continuous lines shown were calculated from fits of each set of data including all [Li^+] to eqn. (3) as described in the Materials and methods section.

groups adjacent to the phosphate (isopropyl phosphate). Propan-1-ol 2-phosphate which has a hydroxyl group adjacent to the phosphate is a substrate, if only a poor one. Interestingly, L-α-glycerophosphate, which also has one adjacent hydroxyl group, is a much better substrate than propan-1-ol 2-phosphate. However, the most active substrate in terms of V and V/K_m was β-glycerophosphate, with a hydroxyl group on each carbon atom adjacent to the phosphate group.

Table 3. Inhibition of *myo*-inositol-1-phosphatase by Li^+

Values in parentheses are the S.E.M. of the estimates of K_i when the data are fitted by a linear least-squares method to eqn. (3). In all cases a minimum of three different Li^+ concentrations plus 0 mM-Li^+ were used to determine K_i.

Substrate	K_i (mM)
D-Ins1*P*	0.61 (±0.04)
2'-AMP	1.70 (±0.12)
β-Glycerophosphate	3.08 (±0.14)
4-Nitrophenyl phosphate	2.78 (±0.18)

In contrast with this series of compounds is 4-nitrophenyl phosphate, with no adjacent hydroxyl groups, which was a substrate, if only a very poor one. In addition, 2'-AMP, which has only one adjacent hydroxyl group, had a higher V than β-glycerophosphate.

Of those compounds that were found not to be substrates, four were monophosphates with no adjacent hydroxyl group, whereas 2',3'-cyclic AMP is a diester. Unlike the rat brain enzyme (Takimoto *et al.*, 1985), the bovine brain enzyme does not appear to utilize phosphoenolpyruvate as a substrate.

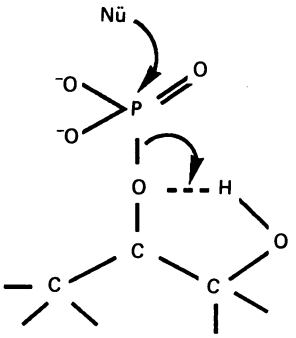
Li^+ inhibition

We tested the enzyme for inhibition by Li^+ with four different substrates, and the results for D-Ins1*P* and 2'-AMP are shown in Fig. 4. Regarding 2'-AMP, 4-nitrophenyl phosphate and β-glycerophosphate, when the slopes of Lineweaver–Burk plots at individual Li^+ concentrations were replotted as a function of [Li^+] according to eqn. (4), the slope of these replots did not significantly differ from zero. In other words, the inhibition by Li^+ is neither competitive nor non-competitive with respect to the substrate, i.e. Li^+ does not bind to the free enzyme. With D-Ins1*P*, however, the slope of the replot made according to eqn. (4) was estimated to be 6.6 ± 4.6 min/μmol. Since, with the other three substrates, no evidence was seen of Li^+ binding to the free enzyme at higher [Li^+] than those used with D-Ins1*P* and, as described in the Materials and methods section, the assays with D-Ins1*P* were less accurate because of the lower volumes used, it is not unreasonable to conclude that in all cases the inhibition by Li^+ is uncompetitive.

The values of K_i obtained from fits of the data to eqn. (3) are shown in Table 3. It is clear that Li^+ was the most potent inhibitor when D-Ins1*P* was the substrate. Even though β-glycerophosphate was a much better substrate than 4-nitrophenyl phosphate, there was little difference in Li^+ inhibition. Li^+, however, was a better inhibitor with 2'-AMP than with β-glycerophosphate, although both substrates have similar V/K_m values. The difference between the two compounds is that 2'-AMP has a higher value of V than does β-glycerophosphate. It may be that the factor in the structure of 2'-AMP that has produced this difference also influences Li^+ binding.

DISCUSSION

The enzyme that we have purified has similar physical properties to those reported for the bovine enzyme in



Nü = nucleophile.

Scheme 2. Acid catalysis by an adjacent hydroxyl group

cruder preparations (Hallcher & Sherman, 1980) and to the rat brain enzyme (Takimoto *et al.*, 1985). The kinetic parameters for catalysis of Ins1*P* dephosphorylation and inhibition by Li^+ obtained for our enzyme preparation are in general agreement with previously published values for the bovine enzyme (Hallcher & Sherman, 1980; Ackerman *et al.*, 1987; Aspley *et al.*, 1987). The bovine brain enzyme appears to have similar substrate specificities to the rat brain enzyme, although we found a higher K_i (0.6 mM) for Li^+ with D-Ins1*P* as a substrate compared with that reported for the rat brain enzyme (0.3 mM) (Takimoto *et al.*, 1985).

The substrate specificities seem to indicate that, in general, at least one hydroxyl group on a carbon atom adjacent to the phosphate is necessary for substrate activity. The exception to this was 4-nitrophenyl phosphate, which displayed low substrate activity. One might conclude from this that the 4-nitrophenyl group is able, to a degree, to perform a similar function to an adjacent hydroxyl group. What the 4-nitrophenyl group can do to aid catalysis is to stabilize the negative charge on the oxygen of the phenolate leaving group. A hydroxyl group on an adjacent carbon atom might be able to do this by acting as an acid catalyst, as shown in Scheme 2, and intramolecular acid catalysis by hydroxyl groups has been reported previously (Kirby & Warren, 1967). This may explain why 2′-AMP exhibits a somewhat higher V than does D-Ins1*P*, since here the adjacent 2′- and 3′-C–O bonds are coplanar. This would enable the formation of a planar five-membered ring system, if the 3′-hydroxyl proton interacted with the 2′-oxygen, which would facilitate proton transfer.

In β-glycerophosphate and D-Ins1*P* both adjacent hydroxyl groups might participate in such acid catalysis, one hydroxyl acting as described above while the other might form a hydrogen bond with one of the phosphate oxygens. On the other hand, one or both of the hydroxyls may simply interact with enzymic amino acid residues so as to place the substrate and active site in the best confirmations for catalysis. The 3-hydroxyl of L-α-glycerophosphate may interact with the active site in this way and thus account for the fact that this compound is a much better substrate in terms of V/K_m than propan-1-ol 2-phosphate, which lacks the second hydroxyl group. If this is the case, this could lend support to the idea that hydroxyl groups distant from the phosphate on Ins1*P* also interact with the active site and thus explain why D-Ins1*P* is a better substrate than β-glycerophosphate.

Ackerman *et al.* (1987) found that *myo*-inositol-1-phosphatase also catalysed the hydrolysis of DL-inositol 4-phosphate and inositol 5-phosphate with similar efficiencies to Ins1*P*, and proposed that the enzyme should really be called *myo*-inositol-monophosphatase. Since we find that the enzyme also utilizes Ins2*P* as a substrate, this means that the enzyme is capable of catalysing the hydrolysis of phosphates on all six positions of the inositol ring. The kinetic parameters for Ins2*P* as a substrate need to be measured with the pure compound. From these values it can then be determined whether the fact that the phosphate is in an axial position in Ins2*P* decreases its potency as a substrate compared with all of the other inositol monophosphates, where the phosphates are equatorial to the inositol ring.

Since the K_i for Li^+ inhibition varies to a degree with substrate structure, provided that with all substrates Li^+ binds to the same enzymic intermediate, this rules out the possibility that Li^+ binds to an E–P_i complex. The fact that Li^+ was an equally good uncompetitive inhibitor of the enzyme with 4-nitrophenyl phosphate as substrate as with β-glycerophosphate, which was a much better substrate, suggests that the fundamental interaction of Li^+ with the enzyme–substrate complex involves only the phosphate group. However, it is clear that other aspects of substrate structure have an effect on Li^+ binding, since with both 2′-AMP and D-Ins1*P* the K_i values were lower for Li^+ inhibition.

In this paper we have described the purification to a high degree of *myo*-inositol-1-phosphatase from bovine brain. We have begun to explore the mechanism of action of the enzyme and its inhibition by Li^+ by examining the effects of substrate structure on these functions. Clearly, much further work is necessary before we have a fuller understanding of these facets of the enzyme.

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