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Research report

Brain inositol monophosphatase identified as a galactose 1-phosphatase

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Abstract

During the course of our analysis of myo-inositol monophosphatase (IMPase), a key enzyme of brain inositol signaling, we found it also hydrolyzes galactose 1-phosphate (Gal 1-P), an intermediate of galactose metabolism. Electrophoretically homogeneous IMPase was prepared from three different sources: (i) bovine brain, (ii) rat brain, and (iii) human brain (recombinant), which demonstrated similar ability to hydrolyze inositol monophosphates and galactose 1-phosphate. The ability of IMPase to use both inositol 1-phosphates and galactose 1-phosphate equally as substrates is of considerable importance in determining lithium's mechanism of action. Our current results suggest that during lithium therapy, both galactose and inositol metabolic pathways can be simultaneously modulated through lithium inhibition of IMPase. Enzyme studies with Mg^{2+} ions as activators and with Li^{+}, Ca^{2+}, Mn^{2+}, Ba^{2+} ions as inhibitors demonstrate that IMPase is a single enzyme possessing the ability to hydrolyze both inositol monophosphates and Gal-1-P with equal efficiency. In addition, gel-filtration chromatographic analysis demonstrated that IMPase and galactose 1-phosphatase activities co-purify in our eletrophoretically homogeneous enzyme preparations. Our results indicate that lithium inhibition of IMPases at clinically relevant concentrations, may modulate both inositol and galactose metabolism, and identifies yet another carbohydrate pathway utilizing IMPase.

Keywords: Inositol monophosphatase; Lithium; Inositol; Galactose 1-phosphatase; Mood-stabilizing drug

1. Introduction

Inositol monophosphatase (IMPase; EC 3.1.3.25) is the key enzyme involved at the distal end of the brain inositol signaling pathway. IMPase hydrolyzes several of the inositol monophosphates generated after receptor stimulation and regenerates free inositol for recycling into inositol phospholipids [1,9,20,27]. The free inositol thus generated by IMPase serves as the precursor source for brain inositol-mediated signaling. Recently, it has been demonstrated that large doses of inositol invoke a positive therapeutic effect in the treatment of depression, [18], panic disorder [3], and obsessive-compulsive disorder [10]. This novel effect of inositol provides new insights into the psychopharmacology and neuropathology of these disorders [31].

Although IMPase is cloned and functionally expressed and its crystal structures deduced [6,7,21], the binding site for lithium can not be identified. However, enzyme studies can offer solutions toward understanding the mechanism of its pharmacological action [14,30]. In this paper, we have shown that IMPase can hydrolyze galactose 1-phosphate (Gal 1-P) with the same extent as those of D-1 and D-3 myo-inositol monophosphates, IMPase's traditional substrates. In addition to dietary source galactose is generated from the turnover of glycoproteins and glycolipids [32] and is critically involved in post-translational galactosylation of several key brain enzymes, proteins, receptors and peptide hormones. The accumulation of large amounts of galactose in galactosemia [12] causes prominent brain damage and high galactose levels may modulate IMPase activity. Here, we demonstrate that IMPase and galactose 1-phosphatase are one and the same enzyme. This observation opens new avenues for investigations of IMPase which controls components of both inositol signaling pathways and galactose metabolism.

Abbreviations: Gal-1P, galactose 1-phosphate; IMPase, inositol monophosphatase; D-1 IP_1, D-myo-inositol 1-phosphate; D-3 IP_1-D-myo-inositol 3-phosphate

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2. Materials and methods

Sprague-Dawley rat brains were obtained from Harlan Bioproducts, Indianapolis, IN, USA. Bovine brains were obtained directly from local abattoirs. D-1 and D-3 myo-inositol mono-phosphates (D-1 IP_1 and D-3 IP_1), electrophoretically homogeneous rat and bovine brain IMPases were isolated as described [26]. In this study all the cations used were of chloride salts.

For routine assays of IMPase and for chromatographic fractions, the method described by Parthasarathy et al. [26] was followed. This assay eliminates alkaline phosphatase activity and is linear through 30 min. The specific activity was expressed in micromoles of phosphate liberated/min/mg protein. Free galactose liberated by IMPase from galactose 1-phosphate was estimated using galactose dehydrogenase [22]. Standard curve was carried out with galactose upto a concentration of 5 mM. IMPase (0.1 ml) was added to solution containing 10 mM Tris-HCl buffer, pH 7.5 and a final concentration of 0.7 mM Gal-1-P (Sigma Chemical Company, St. Louis, MO, USA). Assays were carried out in triplicate so that only 20% of the substrate is hydrolyzed to work within the linear range of protein and time. Protein was assayed using BSA as standard by the method of Lowry et al. [19]. Molecular weight of native IMPase was determined by gel filtration chromatography on TSK-3000 column. Subunit molecular weight was determined by SDS-PAGE using 10–12% gels according to the method of Laemmli [17]. Specific staining of myo-inositol monophosphatase on acrylamide gels after electrophoresis was carried out as described by Parthasarathy et al. [25].

2.1. Human brain IMPase

Human IMPase cDNA (ClonTech, Palo Alto, CA) was cloned into the *NdeI* site of pET-15b plasmid (Novagen). Clones in forward orientation were identified by DNA sequencing. The pET-15b-*IMPase* was transformed into BL-21 (DE3) competent cells and expression induced with 1 mM IPTG. Recombinant bacteria were pelleted by centrifugation and lysed by sonication in 10 volumes of 20 mM Tris HCl buffer, pH 7.8. The homogenate was then cleared by centrifugation at $10\,000 \times g$ for 1 h and the supernatant heated at 80°C for 15 min. IMPase was purified from recombinant bacteria following the method of Parthasarathy et al. [26].

3. Results

3.1. Enzyme studies

The IMPase preparations used in these experiments were derived from bovine brain, rat brain and human brain (recombinant) preparations. Table 1 shows that IMPase prepared from all three sources is capable of hydrolyzing Gal 1-P, in addition to its traditional substrates D-1 IP_1 and D-3 IP_1, at the same rate (*specific activity of rat brain IMPase*: Gal 1-P = 7.8 ± 0.18; D-IP_1 = 7.6 ± 0.08; D-3 IP_1 = 7.7 ± 0.13). Table 1 also confirms that the enzyme activities are unaffected by heating at 80°C for 15 min, demonstrating heat resistance which is characteristic of IMPase [27]. Galactose 6-phosphate was also tested as a substrate for IMPase under standard assay conditions and demonstrated a specific activity of ~5% of that of Gal 1-P and D-1-IP_1 (data not shown).

Table 1
Specific IMPase activities (micromoles of phosphate liberated/min/mg protein) from different sources. The final enzyme preparations were electrophoretically homogeneous as evidenced by a single 29 kDa protein on SDS-PAGE analysis

Enzyme source	D-1-myo-inositol monophosphate	D-3-myo-inositol monophosphate	D-Galactose 1-phosphate
Bovine brain	6.1 ± 0.10	5.9 ± 0.12	6.0 ± 0.14
Human brain	8.1 ± 0.19	8.1 ± 0.18	8.1 ± 0.11
Rat brain	7.6 ± 0.08	7.7 ± 0.13	7.8 ± 0.18
Bovine brain (heated)	5.7 ± 0.16	5.6 ± 0.14	5.7 ± 0.08
Human brain (heated)	7.7 ± 0.32	8.1 ± 0.34	7.7 ± 0.41
Rat brain (heated)	7.2 ± 0.15	7.2 ± 0.16	7.3 ± 0.62

A final concentration of 0.7 mM of substrates was used for the determination of enzyme activity. Each value represents mean ± S.D. of triplicate determinations. Values below the white line are the enzyme activities obtained after heating at 80°C for 15 min. A small decrease in specific activity was observed by non-specific heat inactivation which are usually observed with the pure form of enzymes.

Fig. 1 demonstrates the enzyme activities with concentrations of 0.2–50 mM of Mg^{2+} which confirms that 3 mM of Mg^{2+} is the optimal concentration in this reaction. No activity was observed at zero concentration of Mg^{2+} (data not shown). A prominent feature of brain IMPases is its inhibition by lithium, a psycho-active drug. Li^{+} at concentrations ranging from 0.1 mM to 10 mM clearly inhibited IMPase activity (Fig. 2). The lithium inhibition profile was uncompetitive type (data not shown) and similar for Gal 1-P, D-1 IP_1 and D-3 IP_1.

The inhibitory effects of Ca^{2+}, Mn^{2+} and Ba^{2+} ions on IMPase activity with different concentrations ranging from 0.001 mM to 50 mM were also determined (Figs. 2 and 3). These cations produced a similar inhibition of the IMPase reaction as evidenced by their inhibitory effects in the hydrolysis of both substrates. Experiments with IMPase and D-3 myo-inositol monophosphate demonstrated the same pattern of stimulation with Mg^{2+} and inhibition with Li^{+}, Ca^{2+}, Mn^{2+} and Ba^{2+} (data not shown).

3.2. Electrophoretic analyses

Electrophoretically pure IMPase preparations (single subunit on SDS-PAGE gels) were used in these studies to unequivocally demonstrate their physical association of

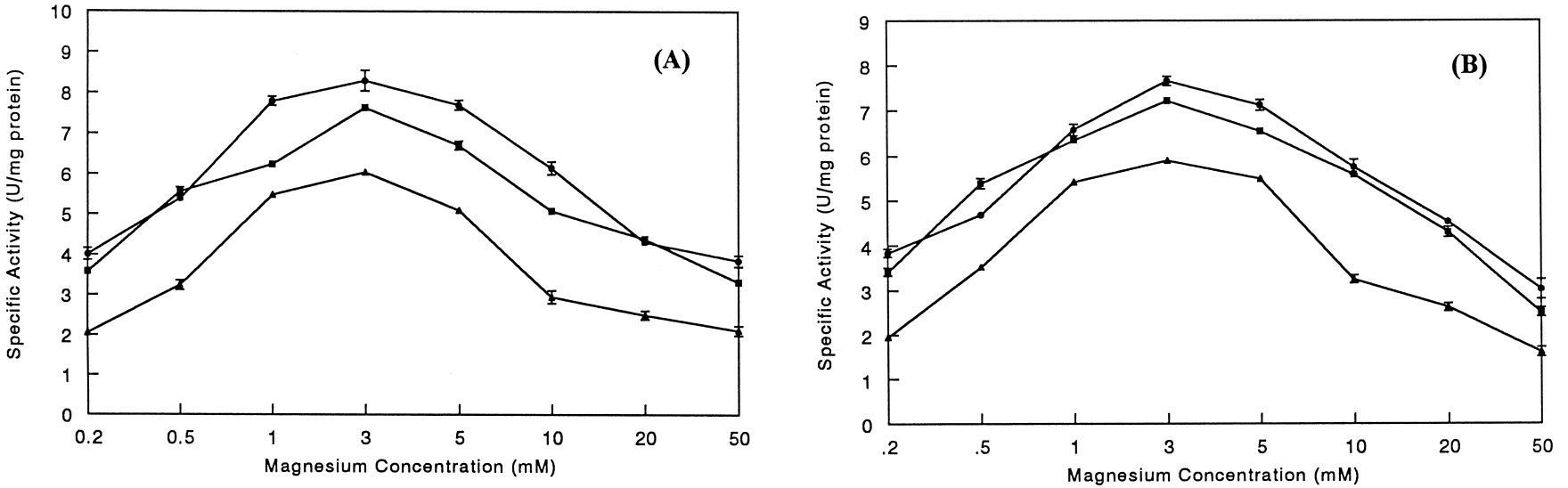

Fig. 1. Effect of Mg^{2+} on IMPase activities of bovine (▲), human (●) and rat (■) brains using D-1-IP (A) and Gal 1-P (B). Each point represents mean ± S.D. (bars) of triplicate determinations.

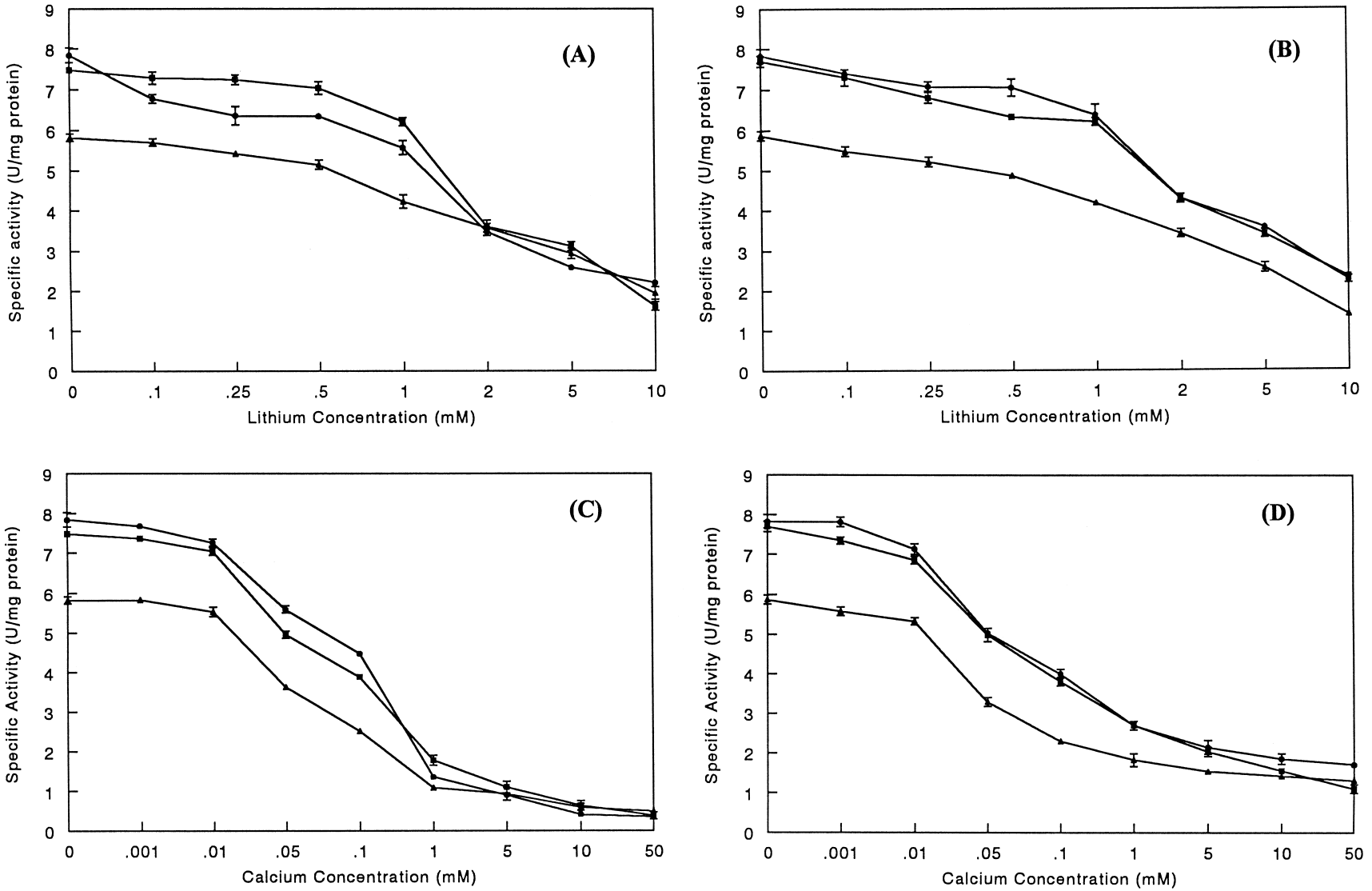

Fig. 2. Effects of Li^+ and Ca^{2+} on IMPase activities of bovine (▲), human (●) and rat (■) brains D-1-IP (A) (C) and Gal 1-P (B) (D), respectively. Each point represents mean ± S.D. (bars) of triplicate determinations.

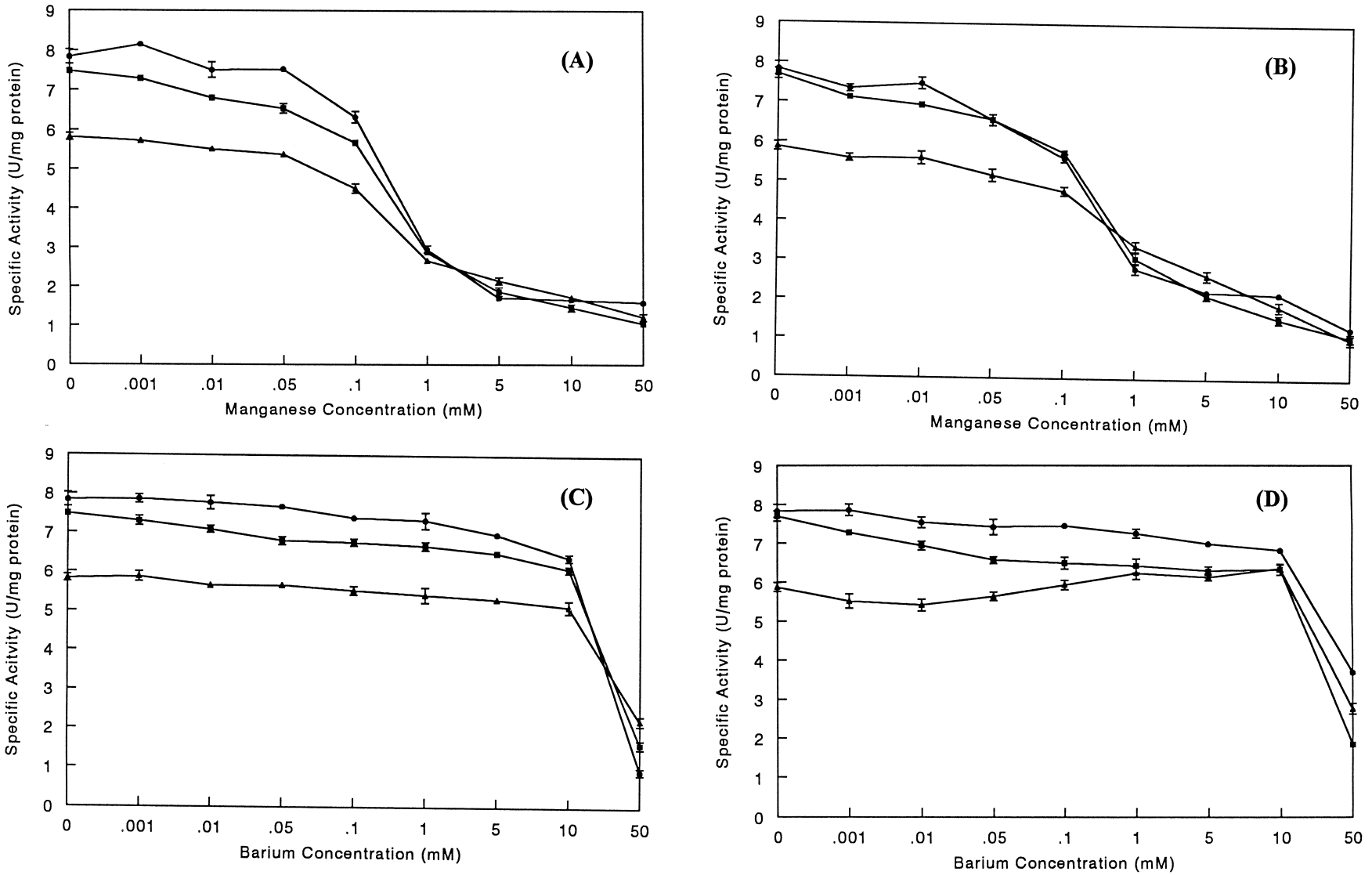

Fig. 3. Effects of Mn^{2+} and Ba^{2+} on IMPase activities of bovine (▲), human (●) and rat (■) brains using D-1-IP (A) (C) and Gal 1-P (B) (D), respectively. Each point represents mean ± S.D. (bars) of triplicate determinations.

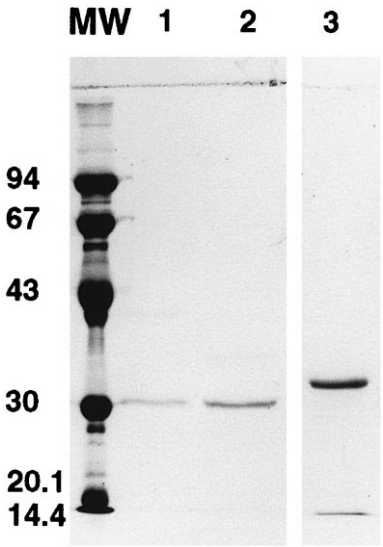

Fig. 4. SDS-PAGE of IMPases from rat, bovine, and human (recombinant) on 10% gel stained with Commassie Brilliant Blue. MW, molecular weight marker proteins (14.4–94 kDa); (1) rat brain; (2) bovine brain; (3) human brain (recombinant). The human recombinant IMPase runs slowly during electrophoresis and is also slightly larger, because it is a fusion protein. Additional twenty amino acids at its amino-terminus derived from an in-frame vector segments of the plasmid did not interfere with functional expression nor its enzyme characteristics.

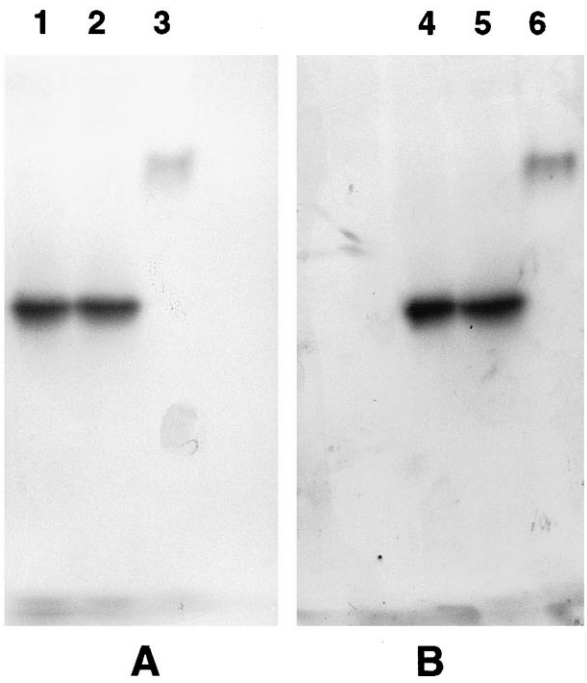

Fig. 5. Specific staining of IMPases with D-1-myo-inositol monophosphate (A) and D-galactose 1-phosphate (B) on native gels. Lanes 1 and 4, rat brain IMPase; 2–5, bovine brain IMPase; and 3–6, recombinant human hippocampal IMPase. A large size of the human enzyme was noticed due to its longer amino-terminus (see legend to Fig. 4).

phosphatase activity to hydrolyze all the three substrates. The presence of the characteristic 29 kDa subunit of IMPase is evident for natural bovine and rat brain enzymes (Fig. 4). The larger subunit size of recombinant human IMPase is due to the longer amino terminus which is derived from an in-frame vector segments of the plasmid pET-15b and this extra twenty amino acids (M-G-S-S-H-

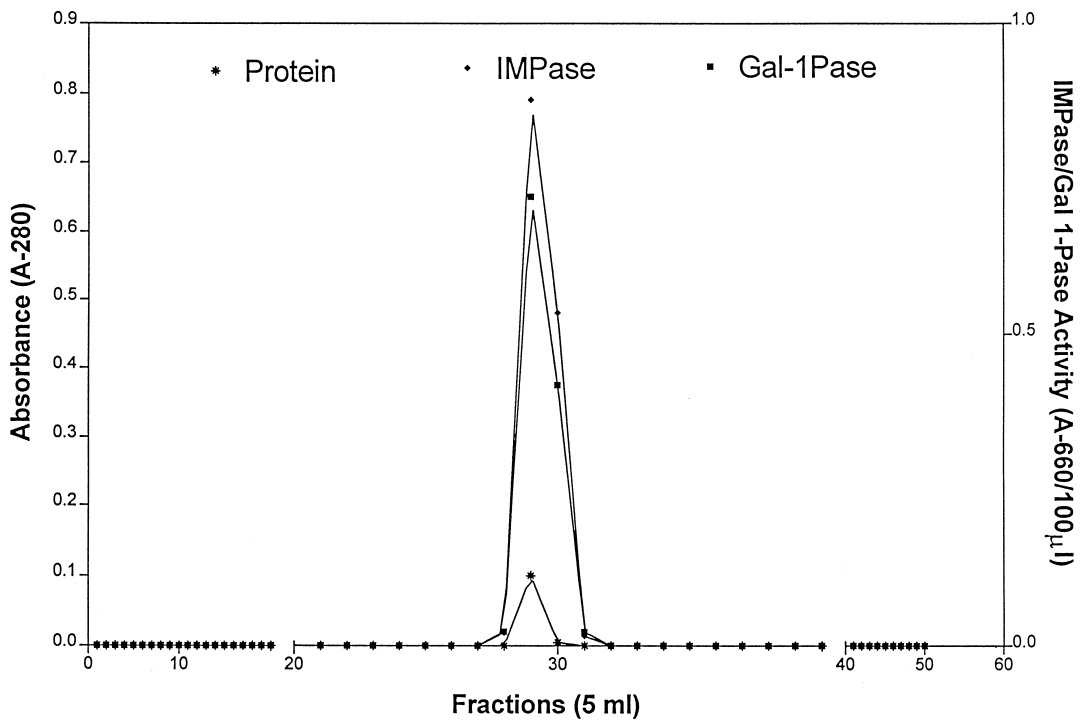

Fig. 6. Gel filtration chromatography of IMPases using TSK-3000 columns. IMPase from bovine brain was injected into the column and 5.0 ml fractions were collected. The activity was carried out using D-myo-inositol phosphates and D-galactose 1-phosphate with a final concentration of 0.7 mM as described in Section 2.

H-H-H-H-H-S-S-G-L-V-P-R-G-S-H) did not reduce the catalytic activity of IMPase (Table 1).

To confirm that the isolated IMPase is a homogeneous phosphatase devoid of any contaminating phosphatases, we carried out specific enzyme staining procedure (Fig. 5) using both D-1 myo-inositol monophosphate and Gal 1-P as substrates. IMPase shows its dimeric native molecular weight of 58 kDa on native gels. After native gel-electrophoresis, incubation in both substrates readily yielded a single green band indicating enzymatic activity in the exact location of enzyme protein.

3.3. Gel-filtration chromatographic analysis

In order to demonstrate unequivocally that IMPase and galactose 1-phosphatase are the same enzyme, we carried out gel filtration chromatography using a TSK-3000 column under non-denaturing conditions. Fig. 6 shows the protein elution pattern of inositol 1-phosphatase, and galactose 1-phosphatase activity of bovine brain enzyme. Both the activities of IMPase and galactose 1-phosphatase were identified in the same fractions (Fig. 6), confirming the gel electrophoresis data (Figs. 4 and 5) and supporting the concept that both enzyme activities are the expression of a single protein.

4. Discussion

While searching for novel cellular substrates for brain IMPase, we found out that Gal 1-P is a substrate of IMPase, equal to the traditional substrates: D-1 and D-3 myo-inositol mono-phosphates. Although, IMPase can be called a ‘specific phosphatase’ because it possesses a unique ability to hydrolyze selective sugar phosphates, unlike ‘non-specific’ alkaline phosphatase which randomly cleaves most monophosphates. Our lab has previously identified other sugar monophosphates that were substrates of IMPase in vitro (D-1 IP1 = D-3 IP1 = 2′-AMP ≫ xylulose 5-P > erythrose 4-P > ribose 5-P > fructose 1-P > fructose 6-P > glucose 6-P > fructose 1,6 bisphosphate [29]. Galactose 1-P is an equivalent substrate to D-1 and D-3 IP1, the functional concept of IMPase must be expanded to include a possible role in vivo in the regulation of brain galactose metabolism. The inhibition of brain IMPase by lithium has focused attention on IMPase as a possible therapeutic site of action in bipolar disorder [1]. IMPase is capable of hydrolyzing galactose 1-phosphate generated by galactokinase [2], a major phosphorylated intermediate of brain galactose metabolism and is essential for post-translational modifications of several proteins, enzymes, receptor complexes and peptide hormones.

The present study with pure IMPase preparations from three different sources (two natural and one recombinantly expressed) confirms that it is capable of hydrolyzing Gal 1-P to the same extent as its primary substrates: D-1 and D-3 inositol monophosphates. The remarkable heat stability property has been used to unequivocally identify IMPase among the other contaminating phosphatases [8]. As demonstrated previously in our laboratory [30] and others [11,16], maximal activity was observed at 3 mM Mg^{2+}, which is optimal for in vitro assays although its physiological levels are in the range of 0.3–0.6 mM [15]. Authenticity of IMPase cleaving different substrates was also substantiated by the same pattern of activation by Mg^{2+} and inhibition by Li^{+}, Ca^{2+}, Mn^{2+} and Ba^{2+}. Hydrolysis of both Gal 1-P and inositol monophosphates were similarly sensitive to both the monovalent and divalent cations mentioned which supports that enzymic activity is mediated only by a single enzyme. Although the effect of lithium on brain galactose metabolism in vivo is presently unknown, it is imperative from the present studies that the level of brain Gal 1-P is controlled by IMPase and the administration of lithium could not only modulate brain inositol signaling pathways, but galactose metabolism as well.

Further studies by SDS-PAGE, specific enzyme staining after native electrophoresis and gel-filtration chromatographic analyses (Figs. 4–6) with bovine brain IMPase confirm that IMPase and galactose 1-phosphatase are one and the same enzyme. Measurements of enzyme activities after column purification established that IMPase activity co-purified with galactose 1-phosphatase activity (Fig. 6). The chromatographic analysis showed that the 58 kDa active protein has catalytic activity toward both substrates. Previously, Gulavita et al. [13] studied some properties of galactose 1-phosphatase in partially purified rat brain preparations. Unfortunately, the naming of this enzyme as galactose 1-phosphatase obscured its ultimate identity, which we now define as lithium-sensitive IMPase. In the same manner several years ago, IMPase was called 2′-nucleotidase due to its ability to hydrolyze 2′-AMP [23]. However, the current name IMPase is more appropriate for this lithium-sensitive brain phosphatase [24].

Although myo-inositol does not have any effect in vitro on IMPase activity [26], a change in brain inositol level (in vivo) may alter a specific pool of inositol as proposed with the ‘inositol depletion hypothesis’ [4,5]. Additionally, lithium inhibition of IMPase may induce a mild ‘galactose depletion’ state. It should be noted that IMPase is a cytosolic enzyme capable of generating free galactose from its phosphorylated form [28]. Although the exact mechanism of intracellular utilization of galactose released by IMPase is uncertain at this time, most probably a small pool of galactose generated by IMPase can only be used for the crucial cellular functions such as galactosylation of receptors and proteins at the post-translational modification steps. The demonstration of Gal 1-P as a major substrate of IMPase demonstrates a new neurochemical pathway to be explored in the search for the therapeutic molecular site of action for lithium, and other mood-stabilizing drugs.

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References

[1] J.R. Atack, H.B. Broughton, S.J. Pollack, Inositol monophosphatase a putative target for Li^+ in the treatment of bipolar disorder, Trends Neurosci. 18 (1995) 343–349.
[2] R.M. Atkinson, R.K. Burton, R.K. Morton, Equilibrium constant of phosphoryl transfer from adenosine triphosphate to galactose in the presence of galactokinase, Biochem. J. 78 (1961) 813–820.
[3] J. Benjamin, J. Levine, M. Fux, A. Aviv, D. Levy, R.H. Belmaker, Double-blind, placebo-controlled, crossover trial of inositol treatment for panic disorder, Am. J. Psychiatry 152 (1995) 1084–1086.
[4] R.H. Belmaker, Y. Bersudsky, G. Agam, J. Levine, O. Kofman, How does lithium work on manic depression? Clinical and psychological correlates of the inositol theory, Annu. Rev. Med. 47 (1996) 47–56.
[5] M.J. Berridge, C.P. Downes, M.R. Hanley, Neural and developmental action of lithium: a unifying hypothesis, Cell 59 (1989) 411–419.
[6] R. Bone, P. Springer, J.R. Atack, Structure of inositol monophosphatase, the putative target of lithium therapy, Proc. Natl. Acad. Sci. USA 89 (1992) 10031–10035.
[7] R.E. Diehl, P. Whiting, J. Potter, N. Gee, C.I. Ragan, D. Linemeyer, R. Schoepfer, C. Bennett, R.A.F. Dixon, Cloning and expression of bovine brain inositol mono-phosphatase, J. Biol. Chem. 265 (1990) 5946–5949.
[8] F. Eisenberg, Jr., R. Parthasarathy, Myo-inositol 1-phosphate, in: H.U. Bergmeyer (Ed.), Methods in Enzymatic Analysis, 3rd ed., Vol. 6, Verlag-Chemie, Weinheim, 1984, pp. 371–375.
[9] S.K. Fisher, A.M. Heacock, B.W. Agranoff, Inositol lipids and signal transduction in the nervous system: an update, J. Neurochem. 58 (1992) 18–38.
[10] M. Fux, J. Levine, A. Aviv, R.H. Belmaker, Inositol treatment of obsessive-compulsive disorder, Am. J. Psychiatry 153 (1996) 1219–1221.
[11] N.S. Gee, C.I. Ragan, K.J. Watling, S. Aspley, R.G. Jackson, G.G. Reid, D. Gani, J.K. Shute, The purification and properties of myo-inositol monophosphatase from bovine brain, Biochem. J. 249 (1988) 883–889.
[12] R. Gitzelmann, Galactose 1-phosphate in the pathophysiology of galactosemia, Eur. J. Pediat. 154 (1995) S45–S49.
[13] S.J. Gulavita, L.-P. Zhang, J. Dougherty, J.A. Dain, Galactose-1-phosphatase in rat brain, J. Neurochem. 57 (1991) 520–526.
[14] L.M. Hallcher, W.R. Sherman, The effects of the lithium ion and other agents on the activity of myo-inositol 1-phosphatase from bovine brain, J. Biol. Chem. 255 (1980) 10896–10901.
[15] F.W. Heaton, Distribution and function of magnesium within the cell, in: N.J. Birch (Ed.), Magnesium and the Cell, Academic Press, Inc., London, 1993, pp. 121–136.
[16] M.P. Honcher, K.E. Ackerman, W.R. Sherman, Chronically administered lithium alters neither myo-inositol monophosphatase activity nor phosphoinositide levels in rat brain, J. Neurochem. 53 (1989) 590–594.
[17] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T_4, Nature 227 (1970) 680–685.
[18] J. Levine, Y. Barak, M. Gonzales, H. Szor, A. Elizur, O. Kofman, R.H. Belmaker, Double-blind, controlled trial of inositol treatment of depression, Am. J. Psychiatry 152 (1995) 792–794.
[19] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin-phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[20] P.W. Majerus, Inositol phosphate biochemistry, Annu. Rev. Biochem. 61 (1992) 225–250.
[21] G. McAllister, P. Whiting, E.A. Hammond, M.R. Knowles, J.R. Atack, F.J. Bailey, R. Maigetter, C.I. Ragan, cDNA cloning of human and rat brain myo-inositol mono-phosphatase, Biochem. J. 284 (1992) 749–754.
[22] W.D.L. Musick, W.W. Wells, Studies on the metabolism of galactose-6-phosphate in rat heart and brain, Arch. Biochem. Biophys. 168 (1975) 302–308.
[23] S. Nakamura, S. Yama, J. Ito, M. Kameyama, Purification and properties of 2′-nucleotidase from mammalian brain, Biochim. Biophys. Acta 568 (1979) 30–38.
[24] R. Parthasarathy, F. Eisenberg Jr., The inositol phospholipids: a stereochemical review of biological activity, Biochem. J. 235 (1986) 313–322.
[25] L. Parthasarathy, T.G. Ramesh, C.S. Shyamala Devi, R. Parthasarathy, Specific staining of myo-inositol 1-phosphatase on polyacrylamide gels after electrophoresis, J. Biosci. 14 (1989) 249–253.
[26] L. Parthasarathy, R.E. Vadnal, T.G. Ramesh, C.S. Shyamala Devi, R. Parthasarathy, Myo-inositol monophosphatase from rat testes: purification and properties, Arch. Biochem. Biophys. 304 (1993) 94–101.
[27] L. Parthasarathy, R.E. Vadnal, R. Parthasarathy, C.S. Shyamala Devi, Biochemical and molecular properties of lithium-sensitive myo-inositol monophosphatase, Life Sci. 54 (1994) 1127–1142.
[28] F. Strasser, P.D. Pelton, A.J. Ganzhorn, Kinetic characterization of enzyme forms involved in metal ion activation and inhibition of myo-inositol monophosphatase, Biochem. J. 307 (1995) 585–593.
[29] R. Vadnal, L. Parthasarathy, R. Parthasarathy, New carbohydrate substrates of lithium-sensitive bovine brain myo-inositol monophosphatase, Neurosci. Res. Commun. 16 (1995) 53–58.
[30] R. Vadnal, R. Parthasarathy, Myo-inositol monophosphatase: diverse effects of lithium, carbamazepine, and valproate, Neuropsychopharmacology 12 (1995) 277–285.
[31] R. Vadnal, L. Parthasarathy, R. Parthasarathy, Role of inositol in the treatment of psychiatric disorders, CNS Drugs 6 (1997) 6–16.
[32] A. White, P. Handler, E.L. Smith, R.L. Hill, I.R. Lehman (Eds.), Principles of Biochemistry, 6th ed. McGraw-Hill Book Company, New York, 1978.