myo-Inositol Monophosphatase: A Challenging Target for Mood Stabilising Drugs

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Abstract: *myo*-Inositol monophosphatase has long been a target for drug discovery. Recent work has given detailed insight into its mechanism and dynamics plus new activities and some novel series of inhibitors. The goal of a bio-available inhibitor for this enzyme, however, remains a major challenge for medicinal chemistry.

INTRODUCTION

The use of mood stabilising drugs for the treatment of bipolar affective disorder has been a mainstay of psychiatric medicine since the first report of the anti-manic effects of lithium more than 50 years ago [1]. The therapeutic mode of action of lithium ions long remained a mystery until Berridge proposed that it acts to suppress the phosphoinositide (PI) signal transduction cascade within neurons [2]. This inositol depletion hypothesis remains unproven yet retains its power. Research into the therapeutic mode of action of Li^+ has recently been reviewed [3-6].

However, lithium therapy is unfortunately accompanied by severe toxicity problems [7] and so a replacement for this most useful treatment with reduced side effects is desirable. The crucial target of Li^+ in the PI signalling cycle is the enzyme *myo*-inositol monophosphatase (IMPase) as this step is common to all the relevant dephosphorylation pathways. Accordingly this protein has been the subject of several major industrial and academic research programmes. Research into the structure and mechanism of this enzyme has been reviewed in the past [5,6,8,9] and the inhibitors of IMPase have also been the subject of review [9-11] but there have been no major reviews on the enzyme itself in nearly ten years and so this review will focus primarily on the literature regarding IMPase published since 1995.

PHYSIOLOGICAL ACTIVITIES OF IMPase

The main importance of IMPase (E.C. 3.1.3.25), a homodimeric protein of 60,000 molecular weight with a cofactor requirement of Mg^{2+}, is its role in the PI cell signalling cycle and its inhibition by Li^+, Fig. (**1**). However, it has recently become apparent that production of *myo*-inositol for use in the PI signalling cycle may not be the only physiological role of this enzyme.

Ramponi *et al.* purified a Zn^{2+}-dependent acid phosphatase enzyme from bovine brain and showed that it not only had the same molecular weight as IMPase but also possessed the same Mg^{2+}-dependent, but Li^+-sensitive, inositol phosphatase activity [12]. In an independent study Fujimoto *et al.* made a similar discovery [13]. Furthermore, cleavage of this protein with endoproteinases gave peptides that would be expected from the cleavage of IMPase itself [12]. The claim that the two activities originated from the same protein was strengthened by the finding that IMPase purified from bovine brain possesses Zn^{2+}-dependent tyrosine phosphatase activity at pH 5.5 and that polyclonal antibodies raised against the tyrosine phosphatase enzyme were shown to also bind to IMPase with high affinity [14].

In addition to the already known broad substrate specificity of IMPase has been added the ability to dephosphorylate several of the carbohydrate-phosphates involved in carbohydrate metabolism. Known substrates include xylulose 5-phosphate, erythrose 4-phosphate, ribose 5-phosphate, fructose 1-phosphate, fructose 6-phosphate, glucose 6-phosphate and fructose 1,6-bisphosphate [15]. It has also been reported that the IMPases purified from human, bovine, and rat brain were all capable of the hydrolysis of galactose-1-phosphate with similar efficiency to inositol-1-phosphate (Ins-1-P) [16]. These results led to the suggestion that IMPase also has a role in the regulation of carbohydrate metabolism. The discovery of a galactose-3-phosphatase from rat liver that possesses lithium sensitive IMPase activity lends weight to the idea that these activities represent multiple roles for this one enzyme within the physiological systems studied [17].

Complementary to the emergence of these new activities, it has also been found that IMPase is just one member of a protein superfamily of Li^+-sensitive, Mg^{2+}-dependent phosphatase enzymes [18]. Despite limited sequence similarity, these enzymes are structurally very similar with common active site architectures [19].

MODE OF ACTION OF IMPase

Given the variability in activities displayed by IMPase and its broad substrate specificity, it is probable that the detailed mechanism of action of this enzyme will vary somewhat according to which substrate is undergoing hydrolysis and the metal atoms that are employed [20]. Most mechanistic work has focussed on D-inositol-1-monophosphate (D-Ins-1-P) hydrolysis and there have been major advances in the understanding of how this occurs in the last ten years.

When last reviewed the proposed mechanism of action of IMPase was as outlined in Fig. (**2**) [21]. It was known from crystallographic and kinetic evidence that at least two metal

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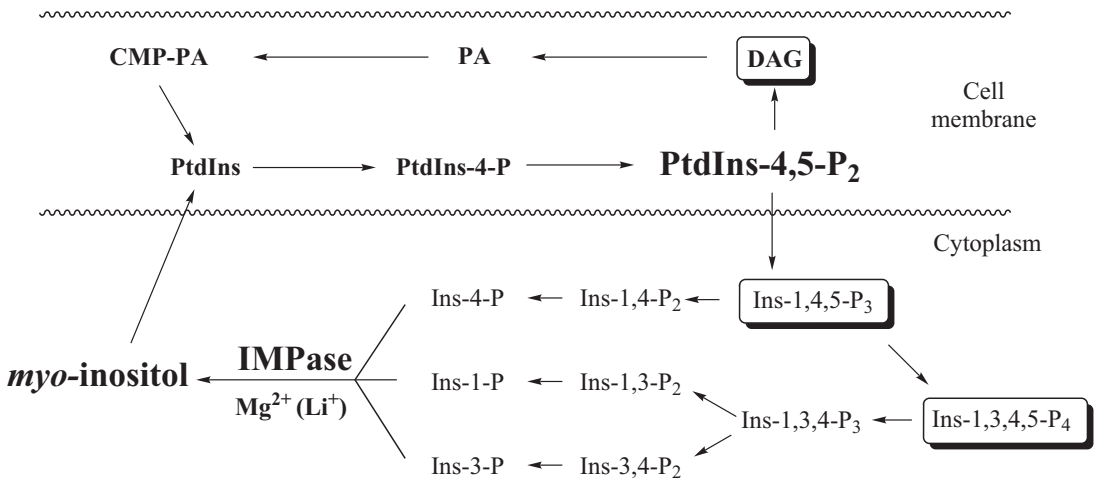

Fig. (1) Overview of the PI cell signal transduction pathway. A G-protein coupled receptor, in response to agonist binding, activates phospholipase C mediated hydrolysis of the membrane-bound lipid phosphatidylinositol-4,5-bisphosphate (PtdIns-4,5-P_2). This gives the second messengers (highlighted with boxes) diacylglycerol (DAG) and *myo*-inositol-1,4,5-trisphosphate (Ins-1,3,4-P_3) that activate downstream cellular responses. Ins-1,4,5-P_3 may be further phosphorylated to give Ins-1,3,4,5-P_4, another second messenger molecule. To inactivate the signal response, each of these second messengers is recycled by the cell. DAG is phosphorylated to give phosphatidic acid (PA) and then ligated to a cytidine to give cytidine monophosphorylphosphatidate (CMP-PA). This can then be attached to a *myo*-inositol molecule to reform phosphatidylinositol (PtdIns) which is then phosphorylated to give PtdIns-4,5-P_2 once more. The *myo*-inositol is generated by a sequence of dephosphorylation reactions which break down Ins-1,3,4-P_3 and Ins-1,3,4,5-P_4 to the parent compound. The final step of this cascade is the breakdown of several inositol monophosphates and is catalysed by Mg^{2+} dependent IMPase. Lithium inhibits several of these dephosphorylation steps *in vivo* including those catalysed by IMPase and so retards the rate of messenger recycling.

ions were needed for catalysis and no phospho-enzyme intermediate could be observed, hence an active site water molecule must be the nucleophilic species that displaces phosphate during the course of the reaction. It was proposed that this water molecule is coordinated to the so-called metal binding site 1 prior to phosphate attack. The Mg^{2+} ion at this site is coordinated to the side chains of Glu70, Asp90 and Thr95 along with the backbone carbonyl oxygen of Ile92. The Mg^{2+} occupying site 2 in this proposal (coordinated to Asp220, Asp90 and the backbone carbonyl of Asp93) was proposed to act as a Lewis acid, stabilising the inositol alkoxide anion leaving group [21].

This mechanistic proposal, however, did not account for all experimental observations that were to emerge. Firstly, it assumed a 2-metal mechanism of action, discounting the presence of 3 Mn^{2+} ions present in a crystal structure published in the same year, because one of these Mn^{2+} ions was displaced by phosphate [22]. Since then, two further crystal structures of IMPase, with three metal ions bound in identical sites have been published [23,24]. The first structure contained both the substrate D-Ins-1-P and three Ca^{2+} ions bound in the active site [23]. The second is a high resolution structure that has three Mg^{2+} ions bound [24]. This latter paper is particular compelling since Mg^{2+} is the natural cofactor involved in the reaction. In addition, the structural features of the active site were super-imposable on those found for similar enzymes [25,26]. Glu70, the phosphate group of Ins-1-P and several solvent molecules, including the proposed nucleophile, coordinate the third metal ion in the active site. Aside from this structural evidence for the involvement of 3 metal ions in catalysis, kinetic arguments in favour of three metal ion catalysis in the hydrolysis of D-Ins-1-P have been presented [23]. Involvement of three metal ions in catalysis is also proposed for two Mg^{2+}-dependent, Li^{+}-sensitive fructose bisphosphatases [27,28]. In addition to this, Ganzhorn *et al.* made the discovery that Lys36, located on a β-sheet near to the active site is important to the catalytic activity of IMPase [29]. It was first noted that IMPase was inactivated by chemical modification using diethylpyrocarbonate during investigations into the importance of histidine residues in IMPase activity [30]. However later work involving similar covalent-inactivation of the enzyme showed that the derivatised residue was Lys36 [29]. It is noteworthy that Atack *et al.* had previously commented that the region encompassing amino acids 36-63 of IMPase may be important to enzyme function [8] and that the N-terminal region of the enzyme had been shown to be important [31]. Interestingly, it was also shown that Lys36 could be protected from inactivation by pre-incubation of the enzyme with the D-Ins-1-P, Mg^{2+} and Li^{+}, presumably locking the enzyme in a conformation that denies the reagents access to Lys36. A K36Q mutant IMPase enzyme was prepared that crystallised in the same conformation as the wild type enzyme, was catalytically active, yet had a 50 fold lower turnover number [29]. It was proposed that Lys36, which is in close contact with Glu70 aids in the polarisation of the water molecule coordinated to the metals at sites 1 and 3 increasing its nucleophilicity and hence the rate of attack at phosphate.

Another problem with the first proposed mechanism [21] was that the role of the 6-OH group of Ins-1-P, known to be essential for a compound to be active as a substrate, was poorly understood at the time. Initially it was assumed that it

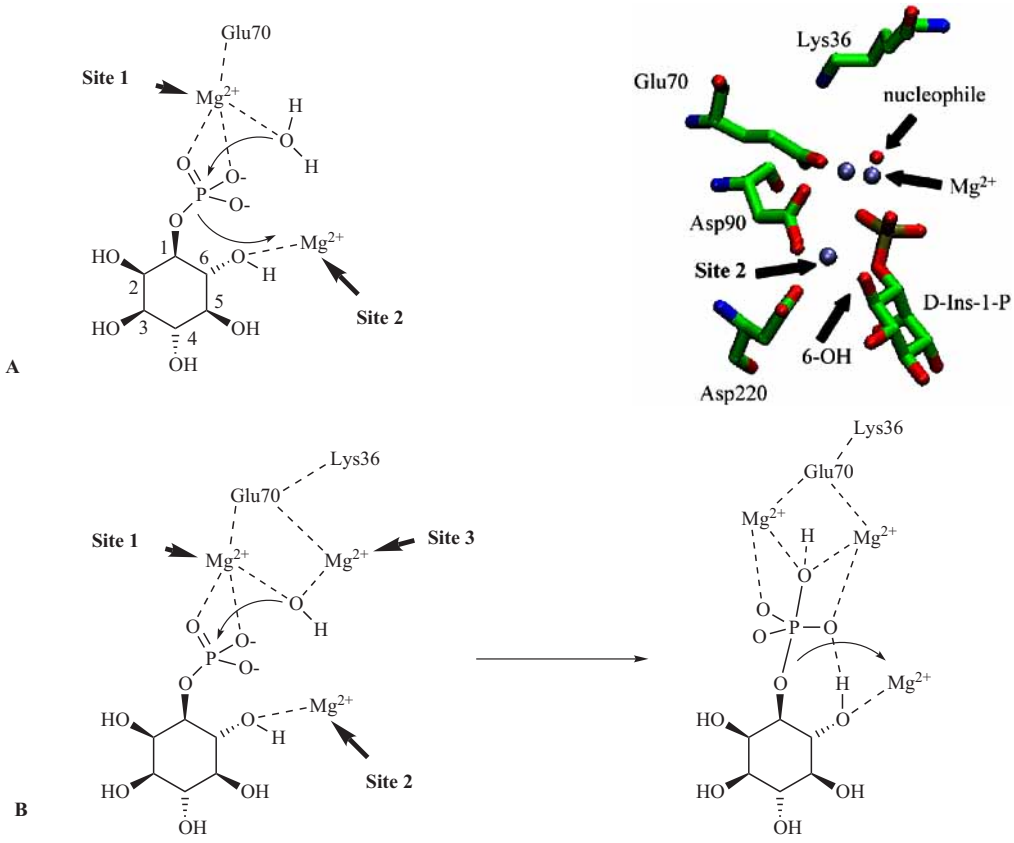

Fig. (2) A. Schematic representation of the mechanism of action of IMPase as proposed by Pollack *et al.* [21]. A two metal dependent reaction required one metal (Site 1) to coordinate the attacking water nucleophile and a second (Site 2) to act as a Lewis acid in stabilising the negative charge that develops on the inositol as it leaves. **B.** Schematic representation of the currently favoured mechanism (Gill *et al.*) [24]. The nucleophilic water molecule is coordinated to two Mg^{2+} ions in sites 1 and 3 and polarisation of this nucleophile is aided by residues Glu70 and Lys36. Stabilisation of the trigonal-bipyramidal transition state is aided by hydrogen bonding to the 6-OH group of the inositol ring. The colour inset shows the key species involved as seen in the crystal structure of Ganzhorn and Rondeau [23].

merely acted as a ligand for the metal ion in site 2, holding it in place to act as a Lewis acid and so stabilise the leaving inositol alkoxide group [21]. However, the preparation and analysis as substrates/inhibitors of a series of Ins-1-P derivatives modified at this position showed that this group acts as a hydrogen bond donor during the hydrolysis of D-Ins-1-P [32,33]. This led to the proposal that the active site nucleophile is not a water molecule bound to metal 1 but in fact is coordinated by both Mg^{2+} at site 2 and hydrogen bonded to the 6-OH group of the substrate (or the equivalent in other substrates). In this scenario, instead of the in-line (S_N2-like) attack of the phosphate group by water proposed initially, there would be an adjacent attack of water from a position perpendicular to the P-O bond that breaks as inositol leaves [34-36]. This latter proposal thus required a pseudorotation of the phosphate group to allow the inositol to occupy an apical position in the pentacoordinate phosphorus transition state. Molecular modelling studies suggested that the in-line and adjacent attack-pseudorotation mechanisms differed little energetically [36]. Use of a chiral phosphate analysis (employing hydrolysis of D-1-S_p-*myo*-inositol [^{17}O]-thiophosphate by IMPase in $H_2{}^{18}O$) showed that the stereochemistry at phosphorus is inverted during hydrolysis and this favoured the in-line attack mechanism as that would lead to inversion whereas the adjacent attack/pseudorotation mechanism would have occurred with retention of configuration [37,38]. A unifying mechanism was therefore proposed because the true role of the 6-OH group of Ins-1-P was still not accounted for. It was suggested that its role, along with that of Mg^{2+} at site 2 must be to hydrogen bond a water molecule that will donate a proton to the inositol alkoxide generated as the phosphate group is cleaved from Ins-1-P [33]. However this was disputed by Gill *et al.* [24] since the 6-OH group of L-Ins-1-P, a better substrate for IMPase than the corresponding D-enantiomer, is too far from the proposed water molecule in their models. Instead it was proposed that the 6-OH group hydrogen bonds to one of the equatorial oxygen atoms in the pentacoordinate transition state formed during the course of the reaction, this stabilises the transition state and so accelerates the reaction [24]. The mechanism of action of IMPase as proposed by Gill *et al.* is illustrated in Fig. (**2**).

NEW INHIBITORS OF IMPase

The importance of IMPase as a drug target is reflected in a myriad of inhibitors that have been prepared and tested over the years. The inhibitors of IMPase studied up to 1999

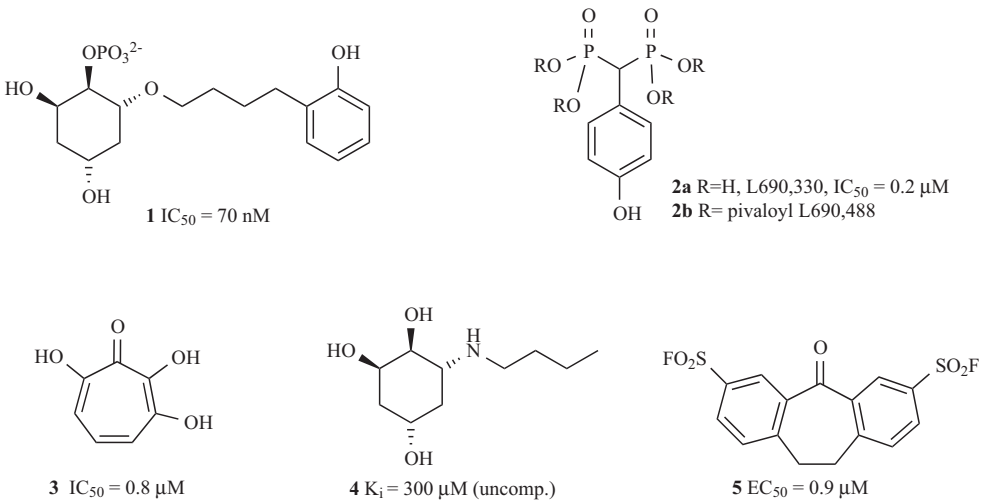

Fig. (3) Some inhibitors of IMPase.

have been reviewed and at this point in time the state of the art was as illustrated by compounds **1** – **3**, Fig. (**3**) [10]. Many analogues of D-Ins-1-P were made and among the most potent of these substrate analogues was compound **1**, possessing a large lipophilic pendant arm at position 6 of the inositol ring. Such compounds retain the phosphate group of the substrate, which limits their usefulness as drugs since they are too polar to cross cell membranes. Modification of this compound at Merck led ultimately to the compound L690,330 (**2a**), which had the stereochemistry of **1** removed and the phosphate replaced with a bisphosphonate group. This compound retained much the same activity against the enzyme but was still too polar for a typical drug [11]. The prodrug of this material L690,488 (**2b**) was active against whole cells but unfortunately did not transport to neurons *in vivo* [11]. Attempts to modify **1** and **2a** to improve their pharmacological properties were reported but the resulting compounds have proved not to inhibit IMPase [39,40]. A major research initiative by Piettre, Ganzhorn and co-workers led to the discovery of a series of α-hydroxytropolone derivatives typified by compound **3** as potent inhibitors of IMPase. Unfortunately, these compounds also failed to transport to the required therapeutic site of action *in vivo* [41,42]. It is now clearly apparent that the preparation of inhibitors of IMPase that can actively transport *in vivo* is a major problem in the search for a drug to replace lithium and most recent work has looked into preparing IMPase inhibitors that will transport more effectively in the body.

Recently a new series of inhibitors of IMPase were reported by our own laboratory which were product mimics rather than the more usual substrate analogues [43,44]. The rationale was that lithium is known to cooperatively bind with phosphate at the active site of the enzyme [45] and it was postulated that these compounds would mimic this behaviour by binding in the presence of phosphate. In addition, removal of the phosphate group would reduce their polarity significantly and so aid transport *in vivo*. These compounds proved to be interesting inhibitors of the enzyme, the best of them prepared to date, compound **4**, was an uncompetitive inhibitor of the enzyme in the absence of phosphate (K_i = 300 μM) yet became a non-competitive inhibitor in its presence [44].

Recent work at Merck has focussed at the discovery of novel IMPase inhibitors with whole-cell activity. To this end a scintillation proximity assay was developed that directly measured inositol phosphate accumulation in cell extracts and applied it to a high throughput screen [46,47]. This led to the discovery of the dibenzosuberone bissulfonylfluoride (**5**) [46,47]. This compound was active in whole cell assays and caused selective accumulation of inositol monophosphates implying that it is a selective inhibitor of IMPase over other inositol phosphatases. The efficacy of this compound was similar to that of Li^+ in the system tested, Fig (**4**)*.

DYNAMICS, STABILITY AND METAL BINDING OF IMPase

The precise mode by which Li^+ inhibits IMPase is still not certain yet examination of this problem has led to some fascinating work regarding the metal binding and dynamics of the enzyme. Leech *et al.* noted that binding of lithium to IMPase was complex in that its mode of inhibition varied with concentration and that cooperative binding with inorganic phosphate occurred [45]. Based on this evidence, combined with the apparent exclusion of divalent cations by lithium from metal binding site 2 in several crystal structures [8], it was proposed that Li^+ inhibits IMPase at therapeutic concentrations by replacement of Mg^{2+} at site 2. It then forms a tight binding complex with inorganic phosphate that is slow to dissociate [8,45]. Strasser *et al.* also studied the kinetics of metal ion activation (and inhibition) of IMPase and came to the same conclusions, showing that Co^{2+} also activated the enzyme towards hydrolysis [20].

The direct observation of Li^+ at its binding site however has proven to be elusive, as Li^+ is too small to be resolved by X-ray crystallography. With their recent 1.4 Å resolution crystal structure of IMPase to hand though, Gill *et al.* propose to use neutron diffraction to solve this problem [24]. A

* Data provided by Dr. Philip E. Brandish, Merck & co., personal communication.

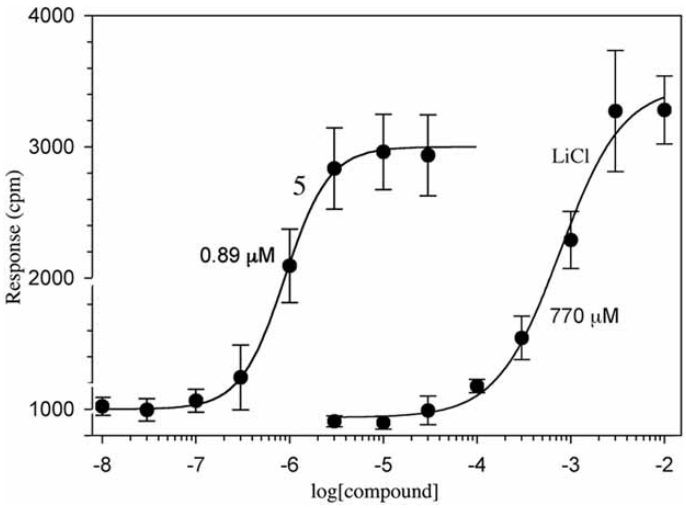

Fig. (4) Dose response curve for dibenzylsuberone bissulfonylfluoride **5** compared to LiCl measured using the scintillation proximity assay of Brandish *et al.* [46,47].

[^{7}Li]-NMR study of IMPase binding of Li^+ concluded that there was only one binding site for Li^+ and the data was also consistent with its location being metal site 2 [48].

The examination of the contribution of Lys36 to the catalytic mechanism of IMPase hints at major conformational changes in the enzyme during the course of the reaction since the presence of metal ions and substrate prevented derivatisation of this amino acid residue [29]. Several groups have investigated potential conformational changes that may occur in IMPase upon metal binding. Thorne *et al*. derivatised IMPase with pyrene maleimide and studied the effects on the fluorescence of pyrene-IMPase upon binding of Mg^{2+} using stopped-flow pre-equilibrium kinetics experiments. Measurement of k_{on} and k_{off} for Mg^{2+} gave a K_d of 450 μM which was in good agreement with results determined by equilibrium binding assays [49]. Furthermore, a slow change in fluorescence was observed, that occurred after the fast initial mixing and was independent of metal binding; when the first 36 N-terminal residues were removed by limited proteolysis, neither the fast nor the slow fluorescence change occurred. It was concluded that binding of Mg^{2+} at the high affinity site (site 1) is accompanied by a pronounced conformational change in the enzyme [49]. Kwon and Churchich used a pyrene-IMPase derivative and an eosin-IMPase derivative to study Mg^{2+}, Co^{2+} and Tb^{3+} binding to IMPase through observation of changes in the fluorescent and phosphorescent spectra upon metal ion binding respectively [50]. The results again gave evidence for structural fluctuation of IMPase upon metal ion binding and also showed that more than one metal ion is required for catalysis [50]. The effects of metal binding upon the CD spectrum of IMPase have also been studied [51]. Spectral perturbation by Mg^{2+} at 275 nm was observed, with a dissociation constant of 275 μM and also at 220 nm with a dissociation constant of 3.9 mM, these were interpreted as indicating structural changes to the enzyme occurring upon Mg^{2+} binding to sites 1 and 2 respectively. The latter binding constant was strengthened four fold by the presence of phosphate [51]. Ca^{2+} and Tb^{3+} also showed perturbations of the CD spectrum in these regions. Li^+ only perturbed the CD spectrum at 220 nm, implying once more that it inhibits the enzyme by binding at metal site 2 [51].

The stability of the so-called metal binding loop has also been studied. It was found that Mg^{2+} stabilised the enzyme against guanidinium hydrochloride induced unfolding as studied by CD spectroscopy [52]. Moreno *et al*. found that in reversible denaturation experiments using guanidinium hydrochloride the unfolded enzyme was still capable of binding Tb^{3+} implying that the enzyme is not truly unfolded even at high concentrations of guanidinium hydrochloride [53]. The case for this is strengthened by the observation that even in 8 M urea, the porcine brain IMPase was still capable of Co^{2+} assisted hydrolysis of p-nitrophenyl phosphate [54].

SUMMARY

In the last ten years there have been some major advances in the research into understanding of the enzyme *myo*-inositol monophosphatase. It seems that its physiological role may not be limited to the PI signal transduction pathway as it also possesses Zn^{2+} tyrosine phosphatase activity and carbohydrate phosphatase activity, the latter suggesting it may also play a role in carbohydrate metabolism.

The mechanism of action of IMPase in inositol phosphate hydrolysis is now known in great detail. It has a cofactor requirement of 3 Mg^{2+} ions two of which are coordinated to a nucleophilic water molecule that attacks the phosphate group. Residues Glu70 and Lys36 are also involved in this step. The final Mg^{2+} ion is a Lewis acid that stabilises the developing negative charge of the leaving inositol alkoxide anion and the role of the 6-OH group of the inositol ring is probably to hydrogen bond the pentacoordinate phosphorus transition state. Metal binding has been shown to cause large conformational changes to this enzyme that can retain some forms of catalytic activity even under strongly denaturing conditions.

The development of new inhibitors has focussed on preparation of inhibitors that are less polar than older generations of inhibitors, a series of product mimics show potential

for future development. A scintillation proximity assay developed by Merck enabled the discovery of IMPase inhibitors with whole cell activity directly.

The ultimate goal of developing a bioavailable inhibitor of IMPase and so a novel drug for bipolar affective disorder has yet to be realised and targeting of this enzyme remains a tough challenge for medicinal chemists yet a deeper understanding of this enzyme's structure and function promises great hope for the future.

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ABBREVIATIONS

Asp	=	Aspartate
CD	=	Circular dichroism
CMP-PA	=	Cytidine monophosphoryl phosphatidate
DAG	=	Diacylglycerol
Glu	=	Glutamate
Ile	=	Isoleucine
IMPase	=	*myo*-Inositol monophosphatase
Ins-1-P	=	*myo*-Inositol 1-phosphate
Ins-3-P	=	*myo*-Inositol 3-phosphate
Ins-4-P	=	*myo*-Inositol 4-phosphate
Ins-1,3-P_2	=	*myo*-Inositol-1,3-bisphosphate
Ins-1,4-P_2	=	*myo*-Inositol-1,4-bisphosphate
Ins-3,4-P_2	=	*myo*-Inositol-3,4-bisphosphate
Ins-1,3,4-P_3	=	*myo*-Inositol-1,3,4-trisphosphate
Ins-1,4,5-P_3	=	*myo*-Inositol-1,4,5-trisphosphate
Ins-1,3,4,5-P_4	=	*myo*-Inositol-1,3,4,5-tetrakisphosphate
Lys	=	Lysine
PA	=	Phosphatidic acid
PI	=	Phosphoinositide
PtdIns	=	Phosphatidylinositol
PtdIns-4-P	=	Phosphatidylinositol-4-phosphate
PtdIns-4,5-P_2	=	Phosphatidylinositol-4,5-bisphosphate
Thr	=	Threonine

REFERENCES

[1] Cade, J. F. J. *Med. J. Aust.* **1949**, *2*, 349.
[2] Berridge, M. J.; Downes, C. P.; Hanley, M. R. *Cell* **1989**, *59*, 411.
[3] Quiroz, J. A.; Gould, T. D.; Manji, H. K. *Mol. Interv.* **2004**, *4*, 259.
[4] Harwood, A. J. *Mol. Psychiatr.* **2005**, *10*, 117.
[5] Atack, J. R. *Brain Res. Rev.* **1996**, *22*, 183.
[6] Atack, J. R.; Broughton, H. B.; Pollack, S. J. *Trends Neurosci.* **1995**, *18*, 343.
[7] Schou, M. *Lithium and the Cell*; Academic Press: London, **1991**.
[8] Atack, J. R.; Broughton, H. B.; Pollack, S. J. *FEBS lett.* **1995**, *361*, 1.
[9] Baker, R.; Broughton, H. B. *Phosphorus Sulfur Silicon Relat. Elem.* **1996**, *109*, 337.
[10] Faroux, C. M. J.; Freeman, S. *J. Enzym. Inhib.* **1999**, *14*, 97.
[11] Atack, J. R. *Med. Res. Rev.* **1997**, *17*, 215.
[12] Caselli, N.; Cirri, P.; Bonifacio, S.; Manao, G.; Camici, G.; Cappugi, D.; Moneti, G.; Ramponi, G. *Biochim. Biophys. Acta* **1996**, *1290*, 241.
[13] Fujimoto, S.; Okano, I.; Tanaka, Y.; Sumida, Y.; Tsuda, J.; Kawakami, N.; Shimohama, S. *Biol. Pharm. Bull.* **1996**, *19*, 882.
[14] Fujimoto, S.; Tsuda, J.; Kawakami, N.; Tanino, H.; Shimohama, S. *Gen. Pharmacol.* **1998**, *31*, 469.
[15] Vadnal, R. E.; Parthasarathy, L.; Parthasarathy, R. *Neurosci. Res. Commun.* **1995**, *16*, 53.
[16] Parthasarathy, R.; Parthasarathy, L.; Vadnal, R. *Brain Res.* **1997**, *778*, 99.
[17] Canales, J.; Buitrago, F.; Faraldo, A.; Avalos, M.; Cameselle, J. C. *Arch. Biochem. Biophys.* **1997**, *343*, 27.
[18] York, J. D.; Ponder, J. W.; Majerus, P. W. *Proc. Nat. Acad. Sci. USA* **1995**, *92*, 5149.
[19] Patel, S.; Martinez-Ripoli, M.; Blundell, T. L.; Albert, A. *J. Mol. Biol.* **2002**, *320*, 1087.
[20] Strasser, F.; Pelton, P. D.; Ganzhorn, A. J. *Biochem. J.* **1995**, *307*, 585.
[21] Pollack, S. J.; Atack, J. R.; Knowles, M. R.; McAllister, G.; Ragan, C. I.; Baker, R.; Fletcher, S. R.; Iverson, L. L.; Broughton, H. B. *Proc. Nat. Acad. Sci. USA* **1994**, *91*, 5766.
[22] Bone, R.; Frank, L.; Springer, J. P.; Atack, J. R. *Biochemistry* **1994**, *33*, 9468.
[23] Ganzhorn, A. J.; Rondeau, J.-M. *Prot. Eng. Suppl. S.* **1997**, *10*, 61.
[24] Gill, R.; Mohammed, F.; Badyal, R.; Coates, L.; Erskine, P.; Thompson, D.; Cooper, J.; Gore, M.; Wood, S. *Acta Crystallogr. D* **2005**, *61*, 545.
[25] Albert, A.; Yenush, L.; Gil-Mascarell, M. R.; Rodriguez, P. L.; Patel, S.; Martinez-Ripoll, M.; Blundell, T. L.; Serrano, R. *J. Mol. Biol.* **2000**, *295*, 927.
[26] Patel, S.; Yenush, L.; Rodriguez, P. L.; Serrano, R.; Blundell, T. L. *J. Mol. Biol.* **2002**, *315*, 677.
[27] Nishimasu, H.; Fushinobu, S.; Shoun, H.; Wakagi, T. *Structure* **2004**, *12*, 949.
[28] Stieglitz, K. A.; Johnson, K. A.; Yang, H.; Roberts, M. F.; Seaton, B. A.; Head, J. F.; Stec, B. *J. Biol. Chem.* **2002**, *277*, 22863.
[29] Ganzhorn, A. J.; Lepage, P.; Pelton, P. D.; Strasser, F.; Vincedon, P.; Rondeau, J.-M. *Biochemistry* **1996**, *35*, 10957.
[30] Pelton, P.; Ganzhorn, A. J. *J. Biol. Chem.* **1992**, *267*, 5916.
[31] Gee, N. S.; Howell, S.; Ryan, G.; Ragan, C. I. *Biochem. J.* **1989**, *264*, 793.
[32] Schulz, J.; Wilkie, J.; Beaton, M. W.; Miller, D. J.; Gani, D. *Biochem. Soc. Trans.* **1998**, *26*, 315.
[33] Miller, D. J.; Beaton, M. W.; Wilkie, J.; Gani, D. *Chembiochem* **2000**, *1*, 262.
[34] Cole, A. G.; Gani, D. *J. Chem. Soc. Perkin Trans. 1* **1995**, 2685.
[35] Cole, A. G.; Wilkie, J.; Gani, D. *J. Chem. Soc. Perkin Trans. 1* **1995**, 2695.
[36] Wilkie, J.; Cole, A. G.; Gani, D. *J. Chem. Soc. Perkin Trans. 1* **1995**, 2709.
[37] Faroux, C. M. J.; Lee, M.; Cullis, P. M.; Douglas, K. T.; Gore, M. G.; Freeman, S. *J. Med. Chem.* **2002**, *45*, 1363.
[38] Faroux, C. M. J.; Lee, M.; Cullis, P. M.; Douglas, K. T.; Freeman, S.; Gore, M. *J. Am. Chem. Soc.* **1999**, *121*, 8385.
[39] Schmitt, L.; Spiess, B.; Schlewer, G. *Tetrahedron Lett.* **1998**, *39*, 4817.
[40] Schmitt, L.; Cavusoglu, N.; Spiess, B.; Schlewer, G. *Tetrahedron Lett.* **1998**, *39*, 4009.
[41] Piettre, S. R.; Ganzhorn, A.; Hoflack, J.; Islam, K.; Hornsperger, J.-M. *J. Am. Chem. Soc.* **1997**, *119*, 3201.
[42] Piettre, S. R.; Andre, C.; Chanal, M.-C.; Ducep, J., -B.; Lesur, B.; Piriou, F.; Raboisson, P.; Rondeau, J.-M.; Schlelcher, C.; Zimmermann, P.; Ganzhorn, A. J. *J. Med. Chem.* **1997**, *40*, 4208.
[43] Surfraz, M. B.-U.; Miller, D. J.; Gani, D.; Allemann, R. K. *Tetrahedron Lett.* **2003**, *44*, 7677.
[44] Miller, D. J.; Surfraz, M. B.-U.; Akhtar, M.; Gani, D.; Allemann, R. K. *Org. Biomol. Chem.* **2004**, *2*, 671.

[45] Leech, A. P.; Baker, G. R.; Shute, J. K.; Cohen, M. A.; Gani, D. *Eur. J. Biochem.* **1993**, *212*, 693.
[46] Brandish, P. E.; Hill, L. A.; Zheng, W.; Scolnick, E. M. *Anal. Biochem.* **2003**, *313*, 311.
[47] Zheng, W.; Brandish, P. E.; Kolodin, D. G.; Scolnick, E. M.; Strulovici, B. *J. Biomol. Screen.* **2004**, *9*, 132.
[48] Saudek, V.; Vincedon, P.; Do, Q.-T.; Atkinson, R. A.; Sklenar, V.; Pelton, P. D.; Piriou, F.; Ganzhorn, A. *Eur. J. Biochem.* **1996**, *240*, 288.
[49] Thorne, M. R.; Greasely, P. J.; Gore, M. G. *Biochem. J.* **1996**, *315*, 989.

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[50] Kwon, O.-S.; Churchich, J. E. *J. Protein Chem.* **1997**, *16*, 1.
[51] Rees-Milton, K.; Thorne, M.; Greasely, P.; Churchich, J.; Gore, M. G. *Eur. J. Biochem.* **1997**, *246*, 211.
[52] Pineda, T.; Thorne, M. J.; Gore, M. G.; Churchich, J. E. *Biochim. Biophys. Acta* **1996**, *1292*, 259.
[53] Moreno, F.; Corrales, S.; Garcia Blanco, F.; Gore, M. G.; Rees-Milton, K. *Eur. J. Biochem.* **1996**, *240*, 435.
[54] Lau, C. K.; Lo, S. C.; Li, W.; Churchich, D. R.; Kwok, F.; Churchich, J. E. *J. Protein Chem.* **1998**, *17*, 789.