Minireview

Structure and mechanism of inositol monophosphatase

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Received 7 December 1994

Abstract Since lithium inhibits IMPase and modulates phosphatidylinositol (PtdIns) cell signalling at therapeutically relevant concentrations (0.5–1.0 mM), IMPase has attracted attention as a putative molecular target for lithium in the treatment of manic depression. IMPase is a homodimer, with each subunit organised in an $\alpha\beta\alpha\beta\alpha$ arrangement of α-helices and β-sheets, and this type of structure seems crucial to the two-metal catalysed mechanism in which an activated water molecule serves as a nucleophile. Lithium appears to inhibit the enzyme following substrate hydrolysis by occupying the second metal binding site before the phosphate group can dissociate from its interaction with the site 1 metal. The understanding of IMPase structure and the mechanism of substrate hydrolysis and lithium inhibition should be useful in the development of novel inhibitors which may prove clinically useful in the treatment of manic depression.

Key words: Inositol monophosphatase; Lithium; Enzyme inhibitor; Enzyme structure; Enzyme mechanism

1. Inositol monophosphatase: Putative therapeutic target for lithium

Although lithium (generally administered as lithium carbonate) is a very effective treatment for manic depression, it nevertheless has appreciable side effects and a narrow range between therapeutic (0.5–1.0 mM) and toxic (>2 mM) plasma lithium concentrations [1]. Consequently, plasma lithium concentrations have to be monitored in patients receiving lithium therapy. Given these limitations, it is possible that compounds which mimic the mechanism of action of lithium might represent novel treatments for manic depression that are devoid of the side effect and toxicity profile which may be due to millimolar concentrations of lithium interfering non-specifically with a number of key physiological functions (e.g. electrolyte homeostasis, ion transport, etc.).

In order to try and develop compounds which mimic the therapeutic actions of lithium, it is first necessary to establish what the mechanism of action of lithium actually is. Unfortunately, although the use of lithium in the treatment of mania was described 46 years ago by the Australian physician John Cade [2], the mechanism by which lithium exerts its therapeutic effects remain unknown. Elucidating this mechanism of action is complicated by the diverse range of biochemical effects reported for lithium [3,4]. Recently, however, research has focused on the role of lithium in the modulation of signal transduction mechanisms [4,5]. Of the variety of effects of lithium on signal transduction mechanisms, the modulation of the phosphatidylinositol (PtdIns)-linked cell signalling pathway (Fig. 1) has attracted most attention, primarily due to the fact that most of the effects of lithium on this pathway occur at therapeutically relevant concentrations of lithium (0.5–1.0 mM). More specifically, it is proposed that lithium inhibits inositol monophosphatase (IMPase; a crucial enzyme in the recycling of inositol from the inositol polyphosphate second messengers (Fig. 1)), thereby causing a depletion of inositol and consequently a reduced synthesis of PtdIns. This in turn ultimately results in an attenuation of the production of intracellular second messengers in response to extracellular stimuli [6]. Consequently, IMPase is an attractive therapeutic target and accordingly has been subjected to detailed structural and mechanistic analysis which will be reviewed in the present article.

2. Sequence of inositol monophosphatase

Mammalian IMPase, which exists as a homodimer of subunit molecular weight of 30 kDa [7], has been purified and sequenced from human, bovine and rat brain, and the amino acid sequence has been found to be 79% identical between these species [8]. In addition, mammalian IMPase shares 75% identity with frog IMPase [9]. However, despite being highly conserved between species the amino acid sequence of IMPase has very little *overall* identity with any known protein.

Nevertheless, more restricted analyses show that regions of mammalian IMPase do share marked *local* similarity with other proteins (Fig. 2). More specifically, the importance of regions corresponding to IMPase amino acids 87–100, 219–233 and 69–72 (motifs A, B and C, respectively) is indicated by the fact that not only do these regions contain most of the key amino acids involved in substrate and metal binding and nucleophile activation (i.e. Glu^{70}, Asp^{90}, Ile^{92}, Asp^{93}, Gly^{94}, Thr^{95} and Asp^{220}) but corresponding regions also occur in inositol polyphosphate 1-phosphatase (IPP; a monomer of 400 amino acids which removes the 1-phosphate group from Ins(1,3,4)P_3 and Ins(1,4)P_2) and fructose 1,6-bisphosphatase (F1,6-BP; a tetramer of subunit size 337 amino acids), both of which, like IMPase, are magnesium-dependent, lithium-sensitive enzymes with similar 2-metal catalysed mechanisms [10–14].

In addition to motifs A, B and C, which contain most of the key amino acids involved in substrate and metal binding and nucleophilic water activation, a region corresponding to amino acids 36–63 is also found in IPP. The fact that this region contains none of the key active site amino acids suggests that the secondary structure of this regions plays an important role in enzyme function.

The occurrence of motifs A, B and C, as well as regions

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SSDI 0014-5793(95)00063-1

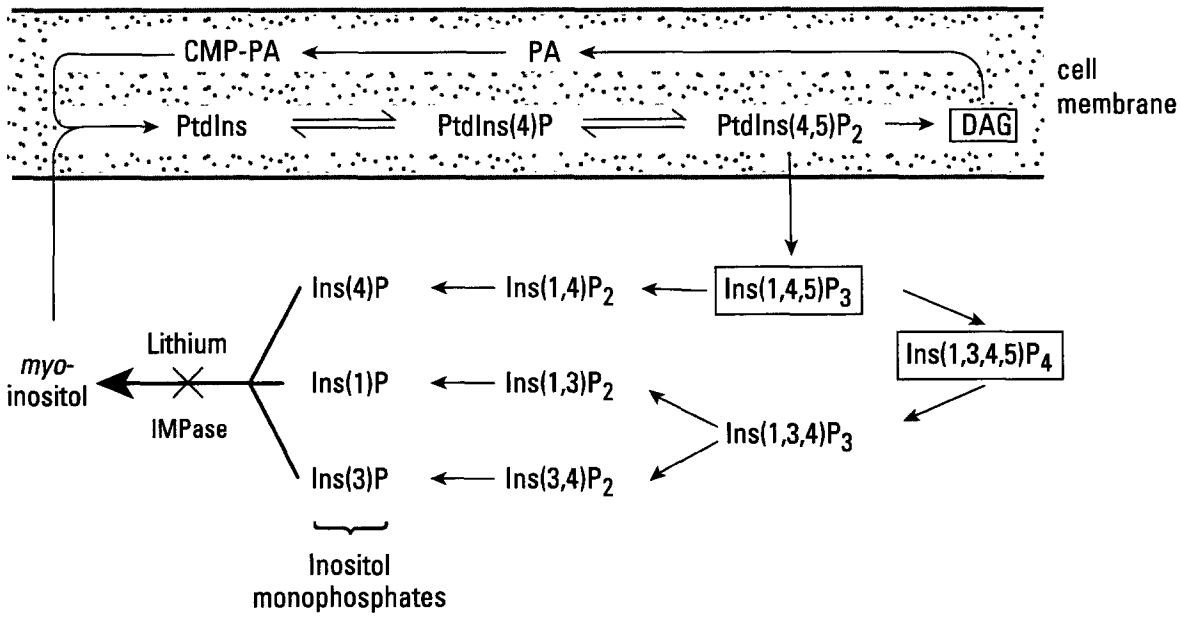

Fig. 1. Simplified representation of the phosphatidylinositol (PtdIns) cell signalling pathway with second messenger molecules highlighted by boxes. Agonist-activated phospholipase C-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate ($PtdIns(4,5)P_2$) produces the second messengers diacylglycerol (DAG) and inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$), which activate protein kinase C and mobilise intracellular calcium, respectively. Whereas the recycling of DAG to the PtdIns precursor cytidine monophosphorylphosphatidate (CMP-PA) is relatively straightforward and proceeds via phosphatidic acid (PA), the metabolism of $Ins(1,4,5)P_3$ is far more complex. Thus, $Ins(1,4,5)P_3$ may be sequentially dephosphorylated to Ins(4)P or alternatively may be phosphorylated to $Ins(1,3,4,5)P_4$ (which itself serves a second messenger function in calcium mobilisation) which is then sequentially dephosphorylated to Ins(1)P or Ins(3)P. The final step in the recycling of inositol from the inositol polyphosphate second messengers is the dephosphorylation of the inositol monophosphates by IMPase. Consequently, in cells without appreciable uptake of extracellular inositol, IMPase plays a crucial role in the provision of inositol required to sustain this signalling pathway.

corresponding to IMPase amino acids 36–63 in the *qa-x* protein of *Neurospora crassa*, the *qutG* protein of *Aspergillus nidulans*, the *suhB* and *cysQ* proteins of *E. coli*. (the latter of which is also known as *amtA*) and the *HAL2/MET22* gene of *Saccharomyces cerevisiae* [9,15–19] suggests that these proteins may be phosphatases. Moreover, some of these proteins (*qa-X*, *qutG* and *suhB*) also have conserved glutamates corresponding to Glu^{213} [17], which is involved in substrate binding (see below).

3. Structure of inositol monophosphatase

In order to examine the three-dimensional structure of IMPase and to identify within the active site the key amino acids involved in substrate binding and the metal-catalysed substrate hydrolysis, crystals of IMPase were prepared for X-ray crystallographic examination. Although the most useful crystal structure would have been an active form of the enzyme (i.e. with magnesium or manganese occupying metal-binding sites) with substrate bound in the active site, this was not possible since, by the very definition that the enzyme is active, the substrate would have been hydrolysed long before the several weeks required for growing of the crystals. Therefore, information was gathered from a number of crystal forms (Table 1).

Each subunit of IMPase occurs as alternating α-helix and β-sheet secondary structures organised in a five-layered αβαβα sandwich. This αβαβα structure also occurs in F1,6-BP and an αβαβ structure has been reported for IPP [10,12,14]. Thus, although there is a lack of overall sequence similarity between IMPase, IPP and F1,6-BP, and they occur as homodimer, monomer and homotetramer, respectively, the key feature of these enzymes that presumably confers their similar properties (i.e. phosphatase activity, magnesium-dependence and inhibition by lithium) is their alternating α-helical–β-sheet tertiary structure. Indeed, superimposition of the structures of these enzymes has identified a common core structure [12]. Furthermore, the region of IMPase between amino acids 36–63, which although not containing any key amino acids is conserved between a number of proteins (Fig. 2), comprises one of the core structural elements common to IMPase, IPP and F1,6-BP [12].

Table 1
Summary of different IMPase crystal complexes

Conditions and resolution (Å)	Comments
No additional ligands (2.5)	Apoenzyme. Information on overall structural features – no direct information on metal or substrate binding.
Gd^{3+}/Li^{+}/sulphate (2.1)	Gd^{3+} binds at Mg^{2+} site 1, sulphate binds at phosphate-binding region, Li^{+} masks second metal-binding site. 9 metal ligands.
Ca^{2+}/Li^{+} (2.6)	Confirms metal site 1 identified using Gd^{3+}.
Gd^{3+}/Li/Ins(1)P or Ins(3)P (2.2–2.3)	Enzyme inhibited by Gd^{3+}, therefore no substrate turnover. Identifies amino acids putatively involved with substrate binding.
Gd^{3+}/Li^{+}/inhibitor (2.8)	Structure with phosphate-containing substrate-based inhibitor confirms binding information from substrate–enzyme structure.
Mn^{2+} (2.6)	Structure in the presence of a metal that supports catalysis. 3 metal sites identified.
Mn^{2+}/phosphate (2.6)	Structure in the presence of a metal that supports catalysis. 2 metal sites identified.

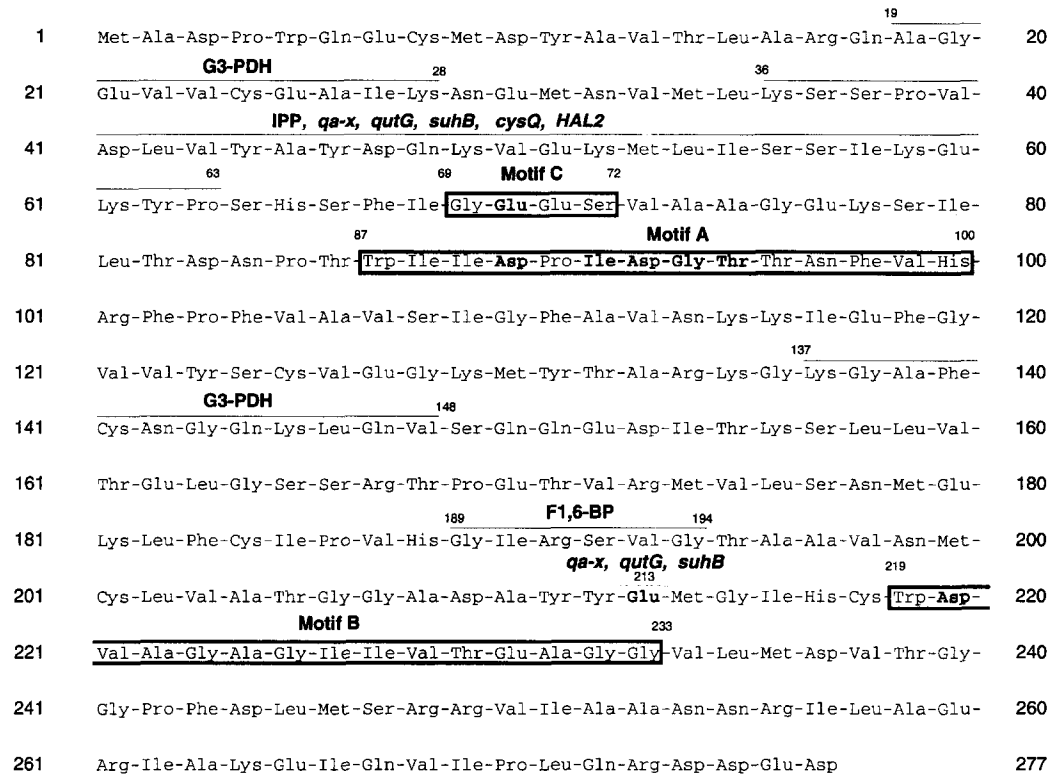

Fig. 2. Sequence of human inositol monophosphatase showing motifs A, B and C along with other regions of local sequence homology. Motifs A, B and C are found in inositol polyphosphate 1-phosphatase (IPP) and fructose 1,6-bisphosphatase (F1,6-BP), indicating a key role for these regions in phosphatase activity. Moreover, the occurrence of motifs A, B and C in the *qa-x* protein of *Neurospora crassa*, the *qutG* protein of *Aspergillus nidulans*, and the *suhB* and *cysQ (amtA)* proteins of *E. coli*, the salt tolerance (halotolerance) gene *HAL2* of *Saccharomyces cerevisiae* (which is identical to the methionine biosynthetic gene *MET22*) along with regions corresponding to IMPase amino acids 36–63 (also found in IPP) and in some cases Glu^{213}, suggests that these proteins, most of which are of unknown function, may possess phosphatase activity. Additional regions possessing homology to cytosolic glycerol 3-phosphate dehydrogenase (G3-PDH) (amino acids 19–28 and 137–148) and F1,6-BP (amino acids 189–194) are also shown. Key amino acids involved in substrate (Asp^{93}, Gly^{94} and Thr^{95}, Asp^{220} and Glu^{213}) and metal (Glu^{70}, Asp^{90}, Ile^{92}, Asp^{93} and Asp^{220}) binding and enzyme mechanism (Glu^{70} and, possibly, Thr^{95}) are highlighted in bold. The nomenclature of motifs A and B is consistent with that of Neuwald et al. [15].

4. Key active site interactions

The structural determination of the IMPase active site permitted the identification of key amino acids involved in putative metal binding, substrate binding and nucleophilic activation interactions (Fig. 3) and formed the framework for interpreting kinetic data derived from site-directed mutagenesis [20,21]. Although some of the key interactions were with the backbone rather than with the side chain of the amino acid (e.g. Ile^{92}, Gly^{94}, Thr^{95} and Ala^{196}, in which case these interactions could not be studied by mutagenesis), most were with amino acid side chains (Table 2) and could be confirmed by mutagenesis [20,21]. In addition, in certain cases hypothesised interactions in IMPase could also be supported by evidence from IPP. For example, on the basis of kinetic data following site-directed mutagenesis, the Thr^{158} of IPP has been proposed to be involved in substrate binding and nucleophilic water activation [12] in a manner analogous to Thr^{95} of IMPase [20,21].

4.1. Metal binding

In the initial report of the structure of IMPase crystallised in the presence of gadolinium (Gd^{3+}), sulphate (which mimics phosphate) and high lithium concentrations [17], and in a subsequent structure crystallised in the presence of Gd^{3+}, Ins(1)P or Ins(3)P and high lithium concentrations [22], only one metal (Gd^{3+})-binding site was identified. However, this proved somewhat misleading since the higher valency of Gd^{3+} involves an additional 2–3 metal binding ligands relative to Mn^{2+} and Mg^{2+}. For example, the Thr^{95} and one of the two metal–sulphate interactions seen in the initial structure [17] were not observed in later crystals [22,23]. It was an unfortunate coincidence that lithium, which is commonly used in the growing of the crystals, was present since it probably occupied the second metal site in these crystals and could not be detected crystallographically. A subsequent structure determined in the presence of only Mn^{2+} revealed the presence of 3 Mn^{2+} in the active site, one of which (ligated by Glu^{70}) was readily displaced upon phosphate binding and was therefore unlikely to be involved in the enzyme mechanism [21]. The crystal structure showed that metal site 1 was ligated by Glu^{70}, Asp^{90} and Ile^{92}, although mutation of Glu^{70} had little effect on metal binding, suggesting that the Glu^{70}–metal 1 interaction was relatively weak [20]. Although the initial structural data had suggested only one (Gd^{3+})-metal binding site, the subsequent manganese or manganese plus

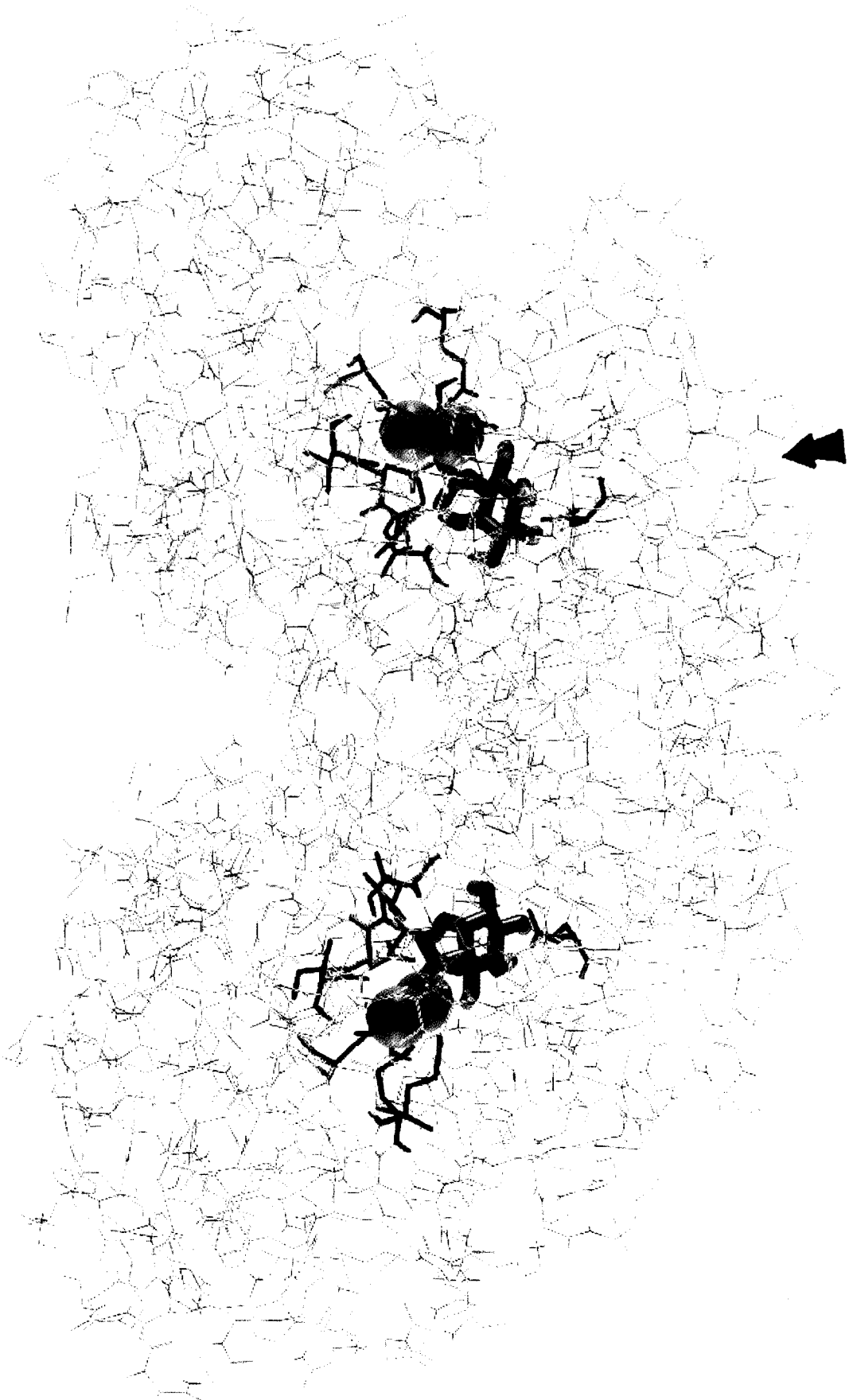

Table 2
Summary of key substrate and metal binding and mechanistic interactions

(A) Summary of involvement of individual amino acids

Residue	Involved in binding to:
Glu^{70}	Site 1 metal, nucleophilic H_2O, site 3 metal
Asp^{90}	Site 1 metal and site 2 metal
Ile^{92*}	Site 1 metal
Asp^{93}	Site 2 metal and substrate 2-OH
Gly^{94*}	Substrate phosphate
Thr^{95*}	Substrate phosphate
Thr^{95}	Nucleophilic H_2O (possibly)
Ala^{196*}	Substrate 2-OH
Glu^{213}	Substrate 4-OH
Asp^{220}	Site 2 metal, substrate 6-OH

(B) Ligands involved with specific functions

Function	Ligands involved and comments
Substrate binding	
Inositol	4-OH binds Glu^{213}. 2-OH binds Asp^{93} and Ala^{196*}. 6-OH binds Asp^{220}.
Phosphate	Gly^{94*}, Thr^{95*}, site 1 metal, 2 waters, residues 95–100 α-helix dipole.
Site 1 metal	Glu^{70}, Asp^{90}, Ile^{92*}, phosphate oxygen, 2 solvent molecules. Binds before substrate and is required for subsequent substrate phosphate binding. Activates H_2O for nucleophilic attack on phosphate phosphorus.
Site 2 metal	Asp^{90}, Asp^{93}, Asp^{220}, substrate ester oxygen, substrate 6-OH. Binds after substrate and acts as a Lewis acid to activate inositol ester oxygen.
Nucleophilic H_2O activation	Site 1 metal, Glu^{70} and, possibly, Thr^{95}.
Lithium inhibition	Binds to phosphate at site 2 metal following substrate hydrolysis.

Functions of individual amino acids was supported by site-directed mutagenesis except for residues denoted by *, in which case interactions were with amide or carbonyl groups of the backbone and therefore could not be altered by mutagenesis.

phosphate and structures clearly demonstrated a second metal-binding site which in conjunction with mutagenesis was shown to be ligated by Asp^{90}, Asp^{93} and Asp^{220} [17,20–23].

4.2. Substrate binding

Although the Gd^{3+}-containing structures were somewhat misleading from a metal-binding point of view (because of the presence of lithium and the different valencies of Gd^{3+} and Mn^{2+} or Mg^{2+}), they nevertheless provided useful information regarding substrate binding. Thus, since the enzyme was totally inhibited by Gd^{3+}, it was possible to obtain crystals of IMPase with substrate (Ins(1)P or Ins(3)P), as well as a substrate-based phosphate-containing inhibitor (2,4-dihydroxy inositol 1-phosphate), and thereby identify key amino acids in the active site involved in substrate binding. Three hydroxyl groups in both Ins(1)P and Ins(3)P (the 2-, 4- and 6-OH of Ins(1)P) make 4 hydrogen bond interactions with the enzyme, explaining the comparable affinity of the enzyme for these substrates [7], although the limited number of inositol hydroxyl–enzyme interactions explains the low affinity of the enzyme for inositol itself [24]. More specifically, the interactions between Ins(1)P and the enzyme are between the 2-OH and Asp^{93} and Ala^{196}, 4-OH and Glu^{213} and the 6-OH and Asp^{220}. The substrate phosphate group is ligated by the backbone amides of Gly^{94} and Thr^{95} as well as the site 1 metal and 2 water molecules. In addition, the phosphate group is positioned at the base of an α-helical dipole of residues 95–100 which also probably contributes to phosphate binding.

4.3. Nucleophilic water activation

The nucleophilic water is activated by the site 1 metal, Glu^{70} and, possibly, Thr^{95}.

5. Mechanism

The issue of whether the nucleophile involved in substrate hydrolysis is a water molecule or an active site amino acid residue has been unclear [24–27]. However, site-directed mutagenesis has been used in conjunction with structural, kinetic and molecular modelling data to identify an activated water molecule rather than an amino acid as the nucleophile [21], in agreement with kinetic data which show the absence of a phospho-enzyme intermediate [24].

Initially, since enzyme structures were identified with either 1, 2 or 3 metals present in the binding site, it was unclear how many metal ions participated in the enzyme mechanism. However, direct experimental evidence from metal titration curves and molecular dynamics modelling of IMPase [21] clearly showed that a 2 (but not 1 or 3)-metal enzyme was consistent with observations derived from structure–activity relationships of substrate-based IMPase inhibitors [28] that the 6-OH of the substrate, which is important for the enzyme mechanism [21,29], co-ordinates the site 2 metal. These results implicating 2 metals in the enzyme mechanism also help explain kinetic data [24,26,30,31]. Additional indirect evidence for a 2-metal mechanism of IMPase comes from the observation that both IPP and F1,6-BP also hydrolyse phosphate groups via a 2-metal mechanism involving nucleophilic attack by water [12–14].

The proposed mechanism for IMPase is summarised in Fig. 4. Briefly, the substrate (in this case Ins(1)P) binds with most of the binding energy being contributed by the substrate phosphate group interacting with the site 1 metal. Next, the site 2 metal binds and this permits the nucleophilic water to attack the substrate phosphorus. Inositol then debinds leaving phosphate ligated to both metals. The site 2 metal then debinds and at this stage phosphate normally debinds from the site 1 metal, regenerating free enzyme ready for the next hydrolytic cycle. However, in the presence of lithium, the site 2 metal binding

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Fig. 3. Substrate (Ins(1)P) is shown (in thick bonds) in the active conformation of IMPase (i.e. with two magnesium ions/active site). This data could not be obtained directly (Ins(1)P would have been hydrolysed by the catalytically active magnesium or manganese-containing enzyme) and is therefore an amalgamation of structural information obtained from the Gd^{3+}-inhibited enzyme plus substrate and active enzyme minus substrate crystal structures (Pollack et al. [21]). Amino acid side chains with major roles in the active site are highlighted. The substrate reaches the active site through a channel that is roughly orientated in the direction shown by the arrow.

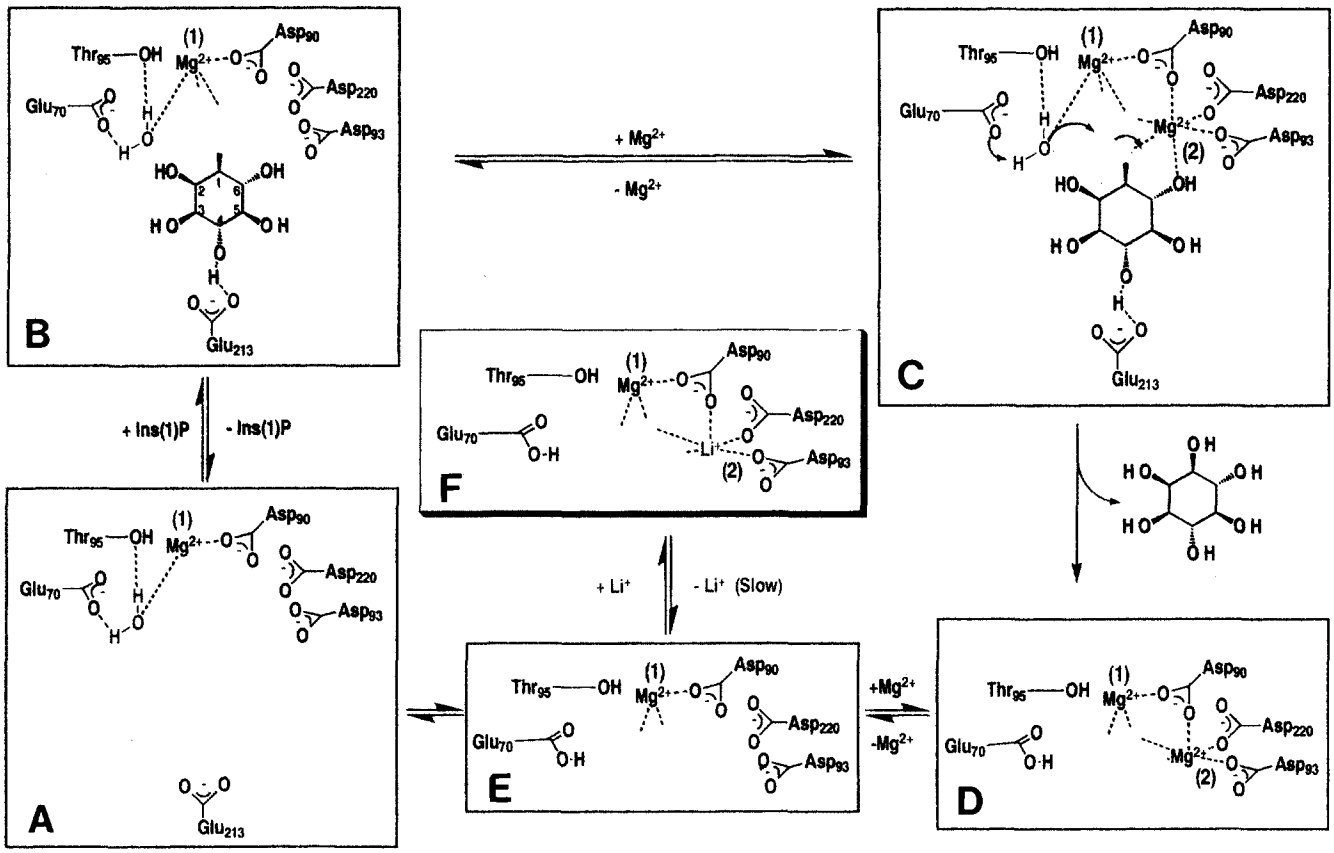

Fig. 4. Schematic representation of the mechanism of IMPase. (A) In the absence of substrate, a single magnesium ion is present in metal site (1). (B) Substrate (inositol 1-phosphate) binds with most of the energy of binding being contributed by the phosphate-magnesium interaction. (C) With substrate bound, a second magnesium ion occupies metal binding site (2). Binding of the second magnesium ion results in an activated water molecule attacking the phosphate phosphorus, resulting in cleavage of the phosphate bond. (D) After inositol debinds, phosphate is ligated by both magnesium ions. (E) The magnesium at metal site (2) rapidly debinds resulting in an enzyme–phosphate complex in which the phosphate is ligated primarily to the metal site (1) magnesium. (F) Ordinarily, the phosphate rapidly debinds regenerating IMPase with one magnesium present ready for the next catalytic cycle. However, in the presence of lithium, the site (2) metal binding site becomes occupied by lithium and phosphate can no longer debind. The enzyme–magnesium–phosphate–lithium complex is then very stable and the enzyme is inhibited.

site becomes occupied by lithium, essentially trapping the phosphate group and thereby inhibiting the enzyme.

6. Conclusions

Structural, kinetic and site-directed mutagenesis data have been used to elucidate the mechanism of IMPase and have demonstrated a 2-metal catalysed hydrolysis of substrate. In this regard, IMPase is similar to F1,6-BP and IPP, both of which are also magnesium-dependent phosphatases that are inhibited by lithium. As regards lithium inhibition, this probably occurs after hydrolysis of the substrate as a consequence of lithium occupying the site 2 metal binding site and thereby forming a stable $E \cdot Mg^{2+} \cdot$ phosphate $\cdot$ Li complex.

Mutagenesis data and sequence comparisons with IPP and F1,6-BP show that key residues in motifs A, B and C are crucial to IMPase activity. However, there is very little sequence similarity outside of these regions and it is the tertiary structure rather than primary amino acid sequence that is the striking common feature of these enzymes. Thus, all three enzymes share a similar organisation of α-helices and β-sheets into alternating layers.

Although substrate-based inhibitors have proved useful tools for evaluating the inositol depletion hypothesis by showing that IMPase inhibitors can mimic the effects of lithium on the PI signalling pathway in vitro [32], these compounds proved unsuitable for in vivo use [28]. In this regard, the combined mechanistic and structural information on IMPase should prove useful in the development of novel inhibitors of this enzyme that may be of clinical utility in the treatment of manic depression.

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