Characterization and regulation of inositol monophosphatase activity in *Mycobacterium smegmatis*

Jérôme NIGOU and Gurdyal S. BESRA¹

Department of Microbiology and Immunology, University of Newcastle, Newcastle upon Tyne, NE2 4HH, U.K.

Mycobacterium tuberculosis and related members of the genus *Mycobacterium* contain a number of inositol-based lipids, such as phosphatidylinositol mannosides, lipomannan and lipoarabinomannan. The synthesis of phosphatidylinositol in *M. smegmatis* is essential for growth and *myo*-inositol is a key metabolite for mycobacteria. Little is known about the biosynthesis of inositol in mycobacteria and the only known *de novo* pathway for *myo*-inositol biosynthesis involves a two-step process. First, cyclization of glucose 6-phosphate to afford *myo*-inositol 1-phosphate via inositol-1-phosphate synthase and, secondly, dephosphorylation of *myo*-inositol 1-phosphate by inositol monophosphatase (IMP) to afford *myo*-inositol. The

following report examines IMP activity in *M. smegmatis* extracts, with regard to pH dependence, bivalent cation requirement, univalent cation inhibition, regulation by growth and carbon source. We show that IMP activity, which is optimal at the end of the exponential growth phase in Sauton's medium, is Mg^{2+} -dependent. Moreover, IMP activity is inhibited by Li^+ and Na^+ , with Li^+ also being able to inhibit growth of *M. smegmatis* *in vivo*. This study represents a first step in the delineation of *myo*-inositol biosynthesis in mycobacteria.

Key words: inositol biosynthesis, kinetics, lithium, magnesium.

INTRODUCTION

Mycobacterium tuberculosis, the causative agent of tuberculosis, continues to be a problem on a worldwide scale, such that the number of deaths attributed to the disease is estimated to be in the order of 3 million deaths per annum ([1]; see also <http://www.who.int/inf-fs/en/fact104.html>). Mycobacteria possess a distinct cell-wall structure, which is thought to play a role both in pathogenicity and as a permeability barrier to conventional antibiotics [2]. The mycobacterial cell wall contains a number of constituents based on *myo*-inositol, such as phosphatidylinositol (PI), phosphatidylinositol mannosides, lipomannan and lipoarabinomannan [2–4]. These lipids play a structural role in the cell wall, but are also considered to be major immunomodulators. Lipoarabinomannan, lipomannan and phosphatidylinositol mannosides have been implicated in the immunopathogenesis of tuberculosis [3,4]. Notably, *M. tuberculosis* lipoarabinomannan is an immunosuppressive molecule inhibiting secretion of the pro-inflammatory cytokines interleukin-12 [5,6] and tumour necrosis factor- α [5], and macrophage activation in response to interferon- γ [7]. Moreover, it has been shown recently that PI is an essential component of the mycobacterial cell wall [8]. Altogether, *myo*-inositol, which is rarely found in prokaryotes [9], represents an important component of these key lipids; however, its biosynthetic origin has not yet been characterized in mycobacteria.

The *de novo* pathway for *myo*-inositol biosynthesis involves basically a two-step process. First, cyclization of glucose 6-phosphate to afford inositol 1-phosphate (I-1-P) via inositol-1-phosphate synthase (IPS; EC 5.5.1.4) and, secondly, dephosphorylation of I-1-P by inositol monophosphatase (IMP; EC 3.1.3.25) to afford inositol [10]. The first enzyme, IPS [11], is essential for growth of yeast and fungi *in vivo* [12]. Recently, using an approach based on tertiary structural prediction analysis, Bachhawat and Mande [13] identified an IPS homologue

(*INO1*) in *M. tuberculosis* (Rv0046c), which was able to functionally complement an *INO1*-deletion mutation in *Saccharomyces cerevisiae*. These results indicated that *M. tuberculosis* Rv0046c probably represents the mycobacterial equivalent of IPS, leading to the first report on the presence of this enzyme in a prokaryotic organism [9]. The completion of the *M. tuberculosis* genome has revealed several putative candidates for IMP activity, but studies examining IMP activities in *M. tuberculosis* have yet to be reported. An *M. smegmatis* mutant with a defective IMP gene homologue (*impA*) has been shown to possess altered cell-envelope permeability properties, with a substantial reduction in the synthesis of phosphatidylinositol dimannoside [14]; however, this study also failed to report IMP or IPS activities in the context of the *impA* mutant [14].

In higher eukaryotes, IMP is an Mg^{2+} -dependent enzyme that is inhibited by lithium salts. As a consequence it is the proposed biological target of lithium in the treatment of manic depression [15]. In hyperthermophilic organisms, IMP provides inositol for the production of small solutes synthesized in response to osmotic or thermal stress [16]. However, in some bacteria, including *Escherichia coli*, inositol-based compounds are undetectable and the role of IMP still remains unclear [17].

In the present study, we have characterized IMP activity in *M. smegmatis* using a simple radiometric assay, thus contributing to the delineation of *myo*-inositol biosynthesis in mycobacteria. Moreover, we show that mycobacterial IMP activity is Mg^{2+} -dependent and inhibited by the univalent cations Li^+ and Na^+ , with Li^+ also being able to inhibit growth of *M. smegmatis* *in vivo*.

MATERIALS AND METHODS

Strains, growth conditions and general chemicals

M. smegmatis mc²155 was a generous gift from Dr W. R. Jacobs, Albert Einstein College of Medicine, New York, NY, U.S.A.

Abbreviations used: I-1-P, inositol 1-phosphate; IPS, inositol-1-phosphate synthase; IMP, inositol monophosphatase; PI, phosphatidylinositol; PNPP, p-nitrophenyl phosphate.

¹ To whom correspondence should be addressed (e-mail g.s.besra@newcastle.ac.uk).

M. smegmatis mc²155 was grown in Sauton's medium supplemented with 0.025% tyloxapol (Sigma) at 37 °C. The effect of glucose as a carbon source on IMP activity was examined by replacing glycerol in Sauton's medium with 2% (w/v) glucose. The effect of *myo*-inositol was examined in the absence or presence of 100 μ M *myo*-inositol in Sauton's medium containing either glycerol or glucose. The effects of LiCl and valproate (2-propyl-pentanoic acid) were examined through the addition of LiCl (10, 25, 50 or 100 mM) and valproate (1, 5, 10 or 50 mM) to the culture medium when the cell cultures reached a D_{600} value of 0.2, and cultures grown for a further 72 h.

Chemicals (reagent grade or better) were purchased from Sigma unless otherwise stated. D-I-1-P was purchased from Fluka. D-[2-³H]I-1-P (16.5 Ci·mmol⁻¹) was purchased from Dupont NEN Research Products.

Preparation of cells extracts

M. smegmatis mc²155 was grown as described above, harvested, washed once with 50 mM Tris/HCl buffer, pH 7.5, and stored at -20 °C until required. Mycobacterial cells (10 g wet weight) were resuspended in 50 mM Tris/HCl buffer, pH 7.5, containing 10 mM MgCl₂ and 5 mM β -mercaptoethanol at 4 °C and subjected to probe sonication (Soniprep 150, 1 cm probe; MSE Sanyo Gallenkamp, Crawley, Sussex, U.K.) for a total time of 10 min in 60 s pulses with cooling intervals of 90 s between pulses. The sonicates were centrifuged at 27000 g for 20 min at 4 °C. The resulting supernatant fraction was centrifuged further at 100000 g for 2 h at 4 °C, yielding membranes and cytosolic (supernatant) fractions. The supernatant was carefully removed and used in enzyme assays. Protein concentrations were determined using the BCA Protein Assay kit (Pierce, Rockford, IL, U.S.A.).

IMP enzyme assays

Standard assay

IMP activity was assayed by measuring the conversion of [³H]I-1-P into [³H]inositol. The reaction mixtures contained, in a total volume of 100 μ l, 0.2 mM [³H]I-1-P (3.7×10^3 c.p.m.·nmol⁻¹), 6 mM MgCl₂ and 25 μ g of protein in 50 mM Tris/HCl, pH 7.5. The reaction mixtures were incubated at 37 °C for 20 min. The reactions were terminated by the addition of 0.9 ml of water and cooled on ice. Inositol and I-1-P were resolved by passage of the reaction mixtures through a 1 ml SAX (strong anion-exchange) column (Whatman). Free inositol was eluted with 3 ml of water, while residual I-1-P remained bound to the SAX column. The total c.p.m. of radiolabelled material eluted from the column was measured by scintillation counting using 10 ml of EcoScintA (National Diagnostics, Atlanta, GA, U.S.A.). IMP activity was determined by subtracting counts present in control assays (either no enzyme or boiled enzyme).

Specific assays

To determine the K_m value for I-1-P, I-1-P concentrations were varied between 0 and 0.2 mM while the Mg²⁺ concentration was maintained at 6 mM. To determine the K_m value for Mg²⁺, Mg²⁺ concentrations were varied between 0 and 6 mM, with the I-1-P concentration being maintained at 0.1 mM. The initial velocities of inositol production were determined after 5 min incubation at 37 °C in 50 mM Tris/HCl, pH 7.5. The effect of pH on IMP activity was determined using the same buffers as described below for the non-specific phosphatase assay.

Non-specific phosphatase assay

Non-specific phosphatase activity was assayed by measuring the conversion of *p*-nitrophenyl phosphate (PNPP) to *p*-nitrophenol. The reaction mixture contained, in a total volume of 100 μ l, 100 μ g of protein, 4 mM PNPP and 6 mM MgCl₂ in one of the following buffers: 50 mM sodium acetate (pH 5.0 or 6.0), 50 mM Tris/HCl (pH 7.0, 7.5, 8.0 or 9.0) or 50 mM glycine/NaOH (pH 10.0) [18]. After an incubation period of 30 min at 37 °C, the reaction was stopped by the addition of 0.6 M NaOH. *p*-Nitrophenol release was determined spectrophotometrically at 405 nm.

RESULTS AND DISCUSSION

Inositol biosynthesis in *M. tuberculosis*

In eukaryotic cells, IMP is involved in both the *de novo* biosynthesis of inositol [19] and phosphoinositide signalling [15]. For bacteria in general, the role of IMP is less clear, since some bacteria possess no PI-based lipids. However, *M. tuberculosis* and related members of the genus *Mycobacterium* contain abundant PI and PI-based lipids. It has been shown that IMP is an essential enzyme in the biosynthesis of PI, since *myo*-inositol is synthesized via *myo*-I-1-P, which is subsequently transformed to 'free' *myo*-inositol in order to act as the acceptor molecule for phosphatidic acid from CDP-diacylglycerol in the generation of PI by the PI synthase [19,20]. Moreover, it has been established in yeast that the biosynthesis of PI is determined primarily by the availability of *myo*-inositol [20,21]. In addition, Jackson et al. [8] determined that the synthesis of PI in *M. smegmatis* was essential for growth, highlighting the importance of *myo*-inositol biosynthesis in *M. tuberculosis*.

Characterization and kinetics of *M. smegmatis* IMP

A crude cytosolic extract was prepared from *M. smegmatis* cultured in Sauton's medium and assayed for IMP activity by a

Figure 1 Characterization of *M. smegmatis* IMP activity in the presence of Mg²⁺ and EDTA

M. smegmatis cells were harvested and a crude cytosolic extract was prepared as described in the Materials and methods section. IMP activity was assayed by measuring the conversion of [³H]I-1-P into [³H]inositol. Briefly, the reaction mixture contained 0.2 mM I-1-P (3.7×10^3 c.p.m.·nmol⁻¹), 6 mM MgCl₂ (■) or 10 mM EDTA (▲) and 12.5–50 μ g of protein in 100 μ l of 50 mM Tris/HCl, pH 7.5.

Figure 2 Mg^{2+} dependence (A) and kinetic analysis of Mg^{2+} dependence (B) for mycobacterial IMP activity

The initial velocities of inositol production were measured with 0.1 mM I-1-P by varying the concentration of Mg^{2+} from 0 to 100 mM (A) or 0 to 6 mM (B). The linear fit (B, inset) was used to calculate the K_m value of Mg^{2+} , which was determined to be 3.2 ± 0.2 mM.

simple radiometric assay measuring the conversion of [3H]I-1-P into [3H]inositol. Our data demonstrate strong IMP activity in *M. smegmatis* (Figure 1). Indeed, this activity is 20-fold higher than that observed in crude extracts prepared from *S. cerevisiae* [22], possibly highlighting the importance of this enzymic activity in mycobacteria. In the presence of increasing concentrations of the bivalent cation, Mg^{2+} , a maximal IMP activity of 1.9 ± 0.1 nmol/min per mg was observed (Figure 2A). IMP activity was abolished completely in the presence of 10 mM EDTA (Figure 1), demonstrating the absolute requirement of bivalent cations for mycobacterial IMP activity. The optimal Mg^{2+} concentration from Figure 2(A) was determined to be 6 mM. The relative Mg^{2+} requirements of *M. smegmatis* IMP are higher than for most mammalian and plant IMP activities, which are typically 1–3 mM [23–26], and similar to that observed for *E. coli* (6 mM) [17], but much less than the optimum value required for IMP activity from thermophilic organisms (50–100 mM) [27,28]. An apparent K_m value of 3.2 ± 0.2 mM was determined for Mg^{2+} (Figure 2B)

Figure 3 pH dependence and specificity of mycobacterial IMP activity

I-1-P dephosphorylation ($\blacktriangle$) was measured at the indicated pH values. Relative activities were normalized to the maximum value obtained at pH 7.5. Non-specific phosphatase activity ($\blacksquare$) was measured at the indicated pH values by assaying PNPP dephosphorylation. Relative activities were normalized to the maximum value obtained at pH 6. There was no correlation between I-1-P and PNPP dephosphorylation, indicating that I-1-P dephosphorylation is specifically catalysed by an IMP.

Figure 4 Kinetic analysis of I-1-P towards IMP activity

The initial velocities of inositol production were measured at 6 mM Mg^{2+} by varying the concentration of I-1-P from 0 to 200 μM . The linear fit was used to calculate the K_m value of I-1-P, which was determined to be 66 ± 15 μM .

with concentrations higher than 6 mM leading to an inhibitory effect (Figure 2A).

The optimal pH for IMP activity was determined as 7.5 (Figure 3). There was no correlation between I-1-P dephosphorylation and residual non-specific phosphatase activity within crude mycobacterial extracts, as assayed by PNPP dephosphorylation [18]. Indeed, as shown in Figure 3, the optimum pH for non-specific phosphatase activity was pH 6.0, and at this pH 20% IMP activity was observed. These data demonstrate clearly that I-1-P dephosphorylation, studied in this report, is specific to an IMP.

The apparent K_m value for I-1-P was determined as 66 ± 15 μM (Figure 4). This value is similar to that observed for bovine brain

Figure 5 Activity of mycobacterial IMP at 0.25 (A) or 6 (B) mM Mg²⁺ as a function of increasing Zn²⁺ (■) and Mn²⁺ (▲) concentration

IMP activity was assayed within crude mycobacterial extracts with 0.1 mM I-1-P in 50 mM Tris/HCl, pH 7.5, at 37 °C. Activities were normalized to the value obtained with 6 mM Mg²⁺ without any other bivalent cation.

(100 μ M) [29] and *E. coli* (64–79 μ M) [17] IMP activities. The enzymic reaction products, phosphate and *myo*-inositol, were both competitive inhibitors with apparent K_i values of 2.7 ± 0.1 and 39 mM, respectively. Phosphate has been described previously as a competitive inhibitor for bovine brain IMP with a K_i value of between 0.22 and 8.0 mM, depending on both pH and Mg²⁺ concentration [29]. In contrast with the competitive inhibitor-type properties of *myo*-inositol with respect to mycobacterial IMP activities, *myo*-inositol was reported to be a poor non-competitive inhibitor of bovine brain IMP, with K_i values of 250 and 400 mM at pH 6.5 and 8.0, respectively [29].

Metal-ion dependence for activation of mycobacterial IMP

To examine whether other bivalent metal ions were able to replace Mg²⁺, a series of experiments was conducted at a low Mg²⁺ concentration (0.25 mM) supplemented with a variety of cations. Zn²⁺ over a range of concentrations (10 nM–10 mM) was not able to activate mycobacterial IMP (Figure 5A). Moreover, at higher Mg²⁺ concentrations (6 mM), Zn²⁺ was a strong inhibitor, with an IC₅₀ of 1 μ M (Figure 5B). These findings are in contrast with those observed for human IMP and Zn²⁺ activation. Indeed, at a concentration of 300 μ M, Zn²⁺ was able to activate human IMP to 50% of the optimal activity obtained with Mg²⁺ [30].

At a low Mg²⁺ concentration (0.25 mM), mycobacterial IMP was active in the presence of Mn²⁺ (Figure 5A), with approx. 25% of the optimal specific activity obtained with 6 mM Mg²⁺.

Figure 6 Inhibition of mycobacterial IMP activity by Li⁺ (◆) and Na⁺ (■)

IMP activity was assayed in crude mycobacterial extracts with 0.1 mM I-1-P in 50 mM Tris/HCl/6 mM MgCl₂, pH 7.5, at 37 °C. Activities were normalized to the value without the univalent cation.

Optimal activity occurred at 100 μ M Mn²⁺ and the apparent K_m for Mn²⁺ was determined to be 23 μ M. As with Mg²⁺, an inhibitory effect was observed at higher Mn²⁺ concentrations, with an IC₅₀ value of 350 μ M. The kinetics of mycobacterial IMP activation by Mn²⁺ were similar to those reported for human IMP (25% optimal specific activity obtained at a concentration of 300 μ M, a K_m value of 50 μ M and an IC₅₀ value of 2 mM) [30]. In experiments conducted at high Mg²⁺ concentrations (6 mM), Mn²⁺ was inhibitory, but less than Zn²⁺, with an IC₅₀ value of 80 μ M. These results are explained by the fact that, at high Mg²⁺ concentrations, Mn²⁺ inhibited the formation of the Mg²⁺-activated enzyme. Increasing Mn²⁺ concentrations finally gave rise to a Mn²⁺-activated enzyme, but with activity lower than the Mg²⁺-activated enzyme (Figure 5B).

Mycobacterial IMP is inhibited by lithium and sodium

Mammalian, yeast and *E. coli* IMP are inhibited by Li⁺, whereas only yeast IMP is inhibited by Na⁺ [31]. As shown in Figure 6, mycobacterial IMP was inhibited by Li⁺ with an IC₅₀ value of 0.8 mM. This value is in the same concentration range as that observed for mammalian IMP (0.6–0.8 mM) [32,33], but slightly less than values obtained for *E. coli* IMP (2 mM) [17] and much higher than values obtained for plants (0.01 mM) [34] and for yeast IMP (0.08 mM) [31]. Inhibition by Li⁺ in the sub-millimolar range is considered as a specificity criterion for IMP. Moreover, dephosphorylation of PNPP was poorly affected by 10 mM Li⁺, as well as 10 μ M Zn²⁺ (results not shown).

Interestingly, mycobacterial IMP was also inhibited by Na⁺ with an IC₅₀ value of 70 mM (Figure 6). The value obtained for Na⁺ is much higher than that observed for Li⁺, but similar to that shown for yeast IMP (80 mM) [31]. Until the present study, inhibition by Na⁺ has never been detected for IMP from any other organism than yeast [31]. Mycobacterial IMP is thus, to our knowledge, the first bacterial IMP shown to be inhibited by Na⁺. It has been proposed that the inhibitory effects of Li⁺ and Na⁺ in enzymes of the IMP family may be explained by differences in the geometry of the active metal site. This feature may now also be extended to the mycobacterial IMP family of enzymes. An insight into the role of *myo*-inositol for mycobacterial metabolism, and the existence of differences between mycobacterial and human IMP, could provide evidence for this enzyme as a potential drug target for anti-mycobacterial therapy.

Figure 7 Mycobacterial IMP activity (■) as a function of whole-cell growth (▲)

Cells were grown in Sauton's medium with 0.025% tyloxapol, harvested at the indicated time points and crude mycobacterial cell extracts prepared. IMP activity was assayed using 0.025 mM I-1-P in 50 mM Tris/HCl/6 mM $MgCl_2$, pH 7.5, at 37 °C.

Figure 8 Effect of lithium on *M. smegmatis* growth

Cells were grown in Sauton's medium without (○) or with 10 (■), 25 (▲) or 50 (△) mM LiCl added at a D_{600} value of 0.2 and growth was monitored for a further 72 h.

Regulation of mycobacterial IMP *in vivo*

It has been reported previously that yeast IMP is regulated by the carbon source (glycerol or glucose), the growth stage and the presence of inositol in the culture medium [22]. In Sauton's medium, which contains glycerol as a carbon source, IMP activity was found to be optimal towards the end of the exponential growth phase (Figure 7). Interestingly, when glucose (20 g/l) was used as the carbon source in Sauton's medium instead of glycerol, the maximum activity was found in the middle of the exponential growth phase (results not shown). These results are in contrast with data obtained with yeast IMP activity, which is activated in the stationary phase in medium containing glucose and *myo*-inositol. The effect of *myo*-inositol in the culture medium was not clear, despite an increase in IMP activity in cells grown in the presence of 100 μ M inositol that was sometimes observed. As found for yeast, *myo*-inositol had no activating effect on mycobacterial IMP activity, suggesting that its putative role may be at the transcriptional level [22]. Moreover, as observed previously, *myo*-inositol had an inhibitory effect on mycobacterial IMP activity by competing with substrate binding.

It has been reported recently that IMP expression in yeast was inhibited by valproate (2-propylpentanoic acid) [35]. *M. smegmatis* cells were subsequently grown with valproate up to a maximum concentration of 50 mM without any significant changes in growth rate (results not shown). The *in vitro* IMP activity in cells grown in the presence of 10 mM valproate was not affected significantly (results not shown). These results are in contrast with the data obtained in yeast, where the presence of 2.5 mM valproate in the culture medium almost completely abolished IMP activity and significantly altered growth characteristics [35]. Altogether, the data demonstrate that yeast and mycobacterial IMP activities are regulated by different mechanisms *in vivo*.

M. smegmatis growth is inhibited by Li^+

Since IMP activity is inhibited by Li^+ , we determined whether *M. smegmatis* growth *in vivo* was also inhibited by Li^+ . LiCl at a concentration of 10 mM significantly affected growth *in vivo*, with a pronounced effect at 50 mM LiCl (Figure 8). The use of 100 mM LiCl almost completely abolished the growth of *M. smegmatis* (results not shown). These inhibitory concentrations are much higher than the *in vitro* IC_{50} value of 0.8 mM obtained earlier for Li^+ (Figure 6). However, extracellular and cytosolic concentrations may differ as a result of Li^+ exclusion by mycobacterial cells.

M. tuberculosis genome and the IMP family of proteins

The present report provides an initial characterization of IMP activity in mycobacterial extracts, with regard to pH dependence, bivalent-cation requirement, univalent-cation inhibition, regulation by growth stage and carbon source. The data represent an important contribution to the delineation of *myo*-inositol biosynthesis in *M. tuberculosis*. The completion of the *M. tuberculosis* genome [36] has revealed several open reading frames that share homology with IMPs. Indeed, Cole et al. [36] identified *Rv1604* (*impA*) as a putative IMP, but further examination suggests at least three other putative candidates, *Rv2701c* (*suhB*), *Rv3137* and *Rv2131c* (*CysQ*), that possess IMP signatures. The apparent redundancy is unexplained. Nevertheless, a plausible explanation may be the importance of *myo*-inositol biosynthesis in mycobacteria. However, some of these gene products may possess other functions. Indeed, SuhB from *E. coli*, which possesses IMP activity, has also been suggested to participate in post-transcriptional control of gene expression [17,37,38]. Moreover, it has been demonstrated recently that the control of gene expression by SuhB is independent of SuhB IMP activity [17]. CysQ from *E. coli* is required for cysteine biosynthesis during aerobic growth [39]. An *E. coli* CysQ mutant can be complemented by the rice *HAL2*-like gene encoding a 3'(2'),5'-diphosphonucleoside 3'(2')-phosphohydrolase [39]. This enzyme regulates the flux of sulphur in the sulphur-activation pathway by converting adenosine 3'-phosphate 5'-phosphosulphate into adenosine 5'-phosphosulphate [39]. It is clear that a better understanding of the role of these different IMP gene homologues in mycobacteria is now required. The present study, which has established basic mycobacterial IMP assays, will facilitate the enzymic study of the above gene products with regard to substrate specificity and kinetic analysis.

This work was supported by the Medical Research Council (Co-operative Group Grant), and grant no. AI-38087 from the National Co-operative Drug Discovery Groups for the treatment of opportunistic infections, National Institutes of Allergy and Infectious Diseases (NIAID), National Institutes of Health.

REFERENCES

- World Health Organization (2000) Tuberculosis, Fact Sheet no. 104, World Health Organization, Geneva
- Brennan, P. J. and Nikaido, H. (1995) The envelope of mycobacteria. *Annu. Rev. Biochem.* **64**, 29–63
- Chatterjee, D. and Khoo, K. H. (1998) Mycobacterial lipoarabinomannan: an extraordinary lipoheteroglycan with profound physiological effects. *Glycobiology* **8**, 113–120
- Vercellone, A., Nigou, J. and Puzo, G. (1998) Relationships between the structure and the roles of lipoarabinomannans and related glycoconjugates in tuberculosis pathogenesis. *Front. Biosci.* **3**, 149–163
- Knutsen, K. L., Hmama, Z., Herrera-Velitz, P., Rochford, R. and Reiner, N. E. (1998) Lipoarabinomannan of *Mycobacterium tuberculosis* promotes protein tyrosine dephosphorylation and inhibition of mitogen-activated protein kinase in human mononuclear phagocytes. Role of the Src homology 2 containing tyrosine phosphatase 1. *J. Biol. Chem.* **273**, 645–652
- Nigou, J., Zelle-Rieser, C., Gilleron, M., Thurnher, M. and Puzo, G. (2001) Mannosylated lipoarabinomannans inhibit IL-12 production by human dendritic cells: evidence for a negative signal delivered through the mannose receptor. *J. Immunol.* **166**, 7477–7485
- Sibley, L. D., Hunter, S. W., Brennan, P. J. and Krahenbuhl, J. L. (1988) Mycobacterial lipoarabinomannan inhibits gamma interferon-mediated activation of macrophages. *Infect. Immun.* **56**, 1232–1236
- Jackson, M., Crick, D. C. and Brennan, P. J. (2000) Phosphatidylinositol is an essential phospholipid of mycobacteria. *J. Biol. Chem.* **275**, 30092–30099
- Bachhawat, N. and Mande, S. C. (2000) Complex evolution of the inositol-1-phosphate synthase gene among archaea and eubacteria. *Trends Genet.* **16**, 111–113
- Chen, I. W. and Charalampous, C. F. (1966) Biochemical studies on inositol. IX. D-Inositol 1-phosphate as intermediate in the biosynthesis of inositol from glucose 6-phosphate, and characteristics of two reactions in this biosynthesis. *J. Biol. Chem.* **241**, 2194–2199
- Majumder, A. L., Johnson, M. D. and Henry, S. A. (1997) 1L-*myo*-inositol-1-phosphate synthase. *Biochim. Biophys. Acta* **1348**, 245–256
- Culbertson, M. R. and Henry, S. A. (1975) Inositol-requiring mutants of *Saccharomyces cerevisiae*. *Genetics* **80**, 23–40
- Bachhawat, N. and Mande, S. C. (1999) Identification of the INO1 gene of *Mycobacterium tuberculosis* H37Rv reveals a novel class of inositol-1-phosphate synthase enzyme. *J. Mol. Biol.* **291**, 531–536
- Parish, T., Liu, J., Nikaido, H. and Stoker, N. G. (1997) A *Mycobacterium smegmatis* mutant with a defective inositol monophosphate phosphatase gene homolog has altered cell envelope permeability. *J. Bacteriol.* **179**, 7827–7833
- Berridge, M. J., Downes, C. P. and Hanley, M. R. (1989) Neural and developmental actions of lithium: a unifying hypothesis. *Cell* **59**, 411–419
- Martin, D. D., Ciulla, R. A. and Roberts, M. F. (1999) Osmoadaptation in archaea. *Appl. Environ. Microbiol.* **65**, 1815–1825
- Chen, L. and Roberts, M. F. (2000) Overexpression, purification, and analysis of complementation behavior of *E. coli* SuhB protein: comparison with bacterial and archaeal inositol monophosphatases. *Biochemistry* **39**, 4145–4153
- Braibant, M. and Content, J. (2001) The cell surface associated phosphatase activity of *Mycobacterium bovis* BCG is not regulated by environmental inorganic phosphate. *FEMS Microbiol. Lett.* **195**, 121–126
- Carman, G. M. and Henry, S. A. (1999) Phospholipid biosynthesis in the yeast *Saccharomyces cerevisiae* and interrelationship with other metabolic processes. *Progr. Lipid Res.* **38**, 361–399
- Nikawa, J. and Yamashita, S. (1997) Phosphatidylinositol synthase from yeast. *Biochim. Biophys. Acta* **1348**, 173–178
- Kelley, M. J., Bailis, A. M., Henry, S. A. and Carman, G. M. (1988) Regulation of phospholipid biosynthesis in *Saccharomyces cerevisiae* by inositol. Inositol is an inhibitor of phosphatidylserine synthase activity. *J. Biol. Chem.* **263**, 18078–18085
- Murray, M. and Greenberg, M. L. (1997) Regulation of inositol monophosphatase in *Saccharomyces cerevisiae*. *Mol. Microbiol.* **25**, 541–546
- Gumber, S. C., Loewus, M. W. and Loewus, F. A. (1984) Further studies on *myo*-inositol-1-phosphatase from the pollen of *lilium longiflorum* Thunb. *Plant Physiol.* **76**, 40–44
- McAllister, G., Whiting, P., Hammond, E. A., Knowles, M. R., Atack, J. R., Bailey, F. J., Maigetter, R. and Ragan, C. I. (1992) cDNA cloning of human and rat brain *myo*-inositol monophosphatase. Expression and characterization of the human recombinant enzyme. *Biochem. J.* **284**, 749–754
- Meek, J. L., Rice, T. J. and Anton, E. (1988) Rapid purification of inositol monophosphate phosphatase from beef brain. *Biochem. Biophys. Res. Commun.* **156**, 143–148
- Parthasarathy, L., Vadnal, R. E., Ramesh, T. G., Shyamaladevi, C. S. and Parthasarathy, R. (1993) *myo*-inositol monophosphatase from rat testes: purification and properties. *Arch. Biochem. Biophys.* **304**, 94–101
- Chen, L. and Roberts, M. F. (1998) Cloning and expression of the inositol monophosphatase gene from *Methanococcus jannaschii* and characterization of the enzyme. *Appl. Environ. Microbiol.* **64**, 2609–2615
- Chen, L. and Roberts, M. F. (1999) Characterization of a tetrameric inositol monophosphatase from the hyperthermophilic bacterium *Thermotoga maritima*. *Appl. Environ. Microbiol.* **65**, 4559–4567
- Leech, A. P., Baker, G. R., Shute, J. K., Cohen, M. A. and Gani, D. (1993) Chemical and kinetic mechanism of the inositol monophosphatase reaction and its inhibition by Li⁺. *Eur. J. Biochem.* **212**, 693–704
- Pollack, S. J., Atack, J. R., Knowles, M. R., McAllister, G., Ragan, C. I., Baker, R., Fletcher, S. R., Iversen, L. L. and Broughton, H. B. (1994) Mechanism of inositol monophosphatase, the putative target of lithium therapy. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 5766–5770
- Lopez, F., Leube, M., Gil-Mascarell, R., Navarro-Avino, J. P. and Serrano, R. (1999) The yeast inositol monophosphatase is a lithium- and sodium-sensitive enzyme encoded by a non-essential gene pair. *Mol. Microbiol.* **31**, 1255–1264
- Hallcher, L. M. and Sherman, W. R. (1980) The effects of lithium ion and other agents on the activity of *myo*-inositol-1-phosphatase from bovine brain. *J. Biol. Chem.* **255**, 10896–10901
- Attwood, P. V., Ducepe, J. B. and Chanal, M. C. (1988) Purification and properties of *myo*-inositol-1-phosphatase from bovine brain. *Biochem. J.* **253**, 387–394
- Gillasp, G. E., Keddie, J. S., Oda, K. and Gruijssem, W. (1995) Plant inositol monophosphatase is a lithium-sensitive enzyme encoded by a multigene family. *Plant Cell.* **7**, 2175–2185
- Murray, M. and Greenberg, M. L. (2000) Expression of yeast INM1 encoding inositol monophosphatase is regulated by inositol, carbon source and growth stage and is decreased by lithium and valproate. *Mol. Microbiol.* **36**, 651–661
- Cole, S. T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., Gordon, S. V., Eiglmeier, K., Gas, S., Barry, 3rd, C. E. et al. (1998) Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature (London)* **393**, 537–544
- Inada, T. and Nakamura, Y. (1995) Lethal double-stranded RNA processing activity of ribonuclease III in the absence of suhB protein of *Escherichia coli*. *Biochimie* **77**, 294–302
- Inada, T. and Nakamura, Y. (1996) Autogenous control of the suhB gene expression of *Escherichia coli*. *Biochimie* **78**, 209–212
- Peng, Z. and Verma, D. P. (1995) A rice HAL2-like gene encodes a Ca²⁺-sensitive 3′(2′),5′-diphosphonucleoside 3′(2′)-phosphohydrolase and complements yeast met22 and *Escherichia coli* cysQ mutations. *J. Biol. Chem.* **270**, 29105–29110

Received 27 July 2001/18 October 2001; accepted 6 November 2001