Human 1-d-my-o-Inositol-3-phosphate Synthase Is Functional in Yeast*

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We have cloned, sequenced, and expressed a human cDNA encoding 1-d-my-o-inositol-3-phosphate (MIP) synthase (hINO1). The encoded 62-kDa human enzyme converted d-glucose 6-phosphate to 1-d-my-o-inositol 3-phosphate, the rate-limiting step for de novo inositol biosynthesis. Activity of the recombinant human MIP synthase purified from Escherichia coli was optimal at pH 8.0 at 37 °C and exhibited $K_M$ values of 0.57 mM and 8 $\mu$m for glucose 6-phosphate and NAD$,^+$, respectively. NH$,^+$ and K$^+$ were better activators than other cations tested (Na$^+$, Li$^+$, Mg$^{2+}$, Mn$^{2+}$), and Zn$^{2+}$ strongly inhibited activity. Expression of the protein in the yeast ino1Δ mutant lacking MIP synthase (ino1Δ/hINO1) complemented the inositol auxotrophy of the mutant and led to inositol excretion. MIP synthase activity and intracellular inositol were decreased about 35 and 25%, respectively, when ino1Δ/hINO1 was grown in the presence of a therapeutically relevant concentration of the anti-bipolar drug valproate (0.6 mM). However, in vitro activity of purified MIP synthase was not inhibited by valproate at this concentration, suggesting that inhibition by the drug is indirect. Because inositol metabolism may play a key role in the etiology and treatment of bipolar illness, functional conservation of the key enzyme in inositol biosynthesis underscores the power of the yeast model in studies of this disorder.

Inositol, a six-carbon cyclitol, is found ubiquitously in biological systems (1–3). myo-Inositol, physiologically the most important stereoisomer of inositol, is the precursor of all inositol-containing compounds including phosphoinositides, inositol phosphates, and cell wall polysaccharides (4). These inositol-containing metabolic products convey signals for a wide variety of hormones, growth factors, and neurotransmitters (5–7), and their metabolism plays a vital role in growth regulation, signal transduction, transcription regulation, membrane biogenesis, and other essential biochemical processes (4, 8, 9).

Inositol metabolism is implicated in the etiology and treatment of bipolar disorder, a severe psychiatric illness that affects 1–2% of the general population (10). Lithium and valproate (VPA)$^1$ are the two FDA-approved drugs for the treatment of bipolar disorder. The cause of this illness is unknown, and the therapeutic mechanisms of the anti-bipolar drugs have not been elucidated. The possibility that inositol metabolism is involved in the etiology of bipolar disorder is supported by findings of aberrant inositol metabolism in bipolar patients (11). Reduced frontal cortex inositol levels and reduced levels of phosphatidylinositol bisphosphate in platelet membrane from bipolar patients have been reported (12–14). In addition, inositol incorporation into membrane phosphoinositides of lymphoblastoid cell lines derived from bipolar patients is reduced (15).

The inositol depletion hypothesis has been proposed to explain the therapeutic mechanism of Li (16, 17) based on the observed uncompetitive inhibition of inositol monophosphatase by Li (16, 18) resulting in decreased inositol and down-regulation of the phosphoinositide cycle. An argument raised against the inositol depletion hypothesis is that VPA does not affect inositol monophosphatase (19), which is, thus, not a common target of the drugs. However, we have shown that VPA decreases intracellular inositol in yeast (20), although it does not inhibit inositol monophosphatase (21). Similar to what is found in yeast, a reduced level of inositol also was reported in rodent brain after acute$^6$ and chronic (23) administration of VPA.

Although a number of metabolic routes emanate from myo-inositol, de novo synthesis of inositol is carried out by one set of enzymatic reactions in all organisms studied to date. d-Glucose 6-phosphate (G-6-P) is converted to 1-d-my-o-inositol-3-phosphate (MIP), which is subsequently dephosphorylated to myo-inositol. The rate-limiting step in inositol de novo biosynthesis is the conversion of G-6-P to MIP, a three-step reaction catalyzed by the enzyme 1-d-my-o-inositol-3-phosphate synthase (EC 5.5.1.4), including an oxidation step with NAD$^+$ serving as a hydrogen acceptor, an intramolecular aldol cyclization step, and finally a reduction step with NADH acting as the hydrogen donor regenerating NAD$^+$ (1). MIP synthase has been purified or partially purified and characterized from a wide range of organisms, and its active form is a multimer of identical subunits ranging in molecular mass from 58 to 67 kDa (1–3, 24–28). The structural gene (INO1) encoding the MIP synthase subunit was first identified and cloned in Saccharomyces cerevisiae (27). Homologs of this gene subsequently have been cloned from several prokaryotic and eukaryotic microorganisms and higher plants (1, 3, 29, 30).

The best studied MIP synthase is the enzyme from S. cerevisiae in which expression has been characterized at the molecular level. INO1 expression is regulated by precursors of phospho-6-phosphate; MIP, 1-d-my-o-inositol 3-phosphate; hINO1, human INO1; opi, overproduction of inositol.

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¶ The abbreviations used are: VPA, valproate; G-6-P, d-glucose

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2 G. Shaltiel, A. Shamir, J. Shapiro, D. Ding, E. Dalton, M. Bialer, A. J. Harwood, R. H. Belmaker, M. L. Greenberg, and G. Agam, submitted for publication.
phospholipid biosynthesis, inositol and choline (31–36). INO1 is fully repressed in the presence of inositol and choline and derepressed more than 10-fold in their absence. Derepression of INO1 when inositol is limiting is brought about by the positive transcriptional activators Ino2p and Ino4p. In the presence of inositol, the negative regulator Opi1p represses transcription of the INO2 gene (37). Because inositol metabolism is so well characterized in yeast, we have utilized this model system to elucidate the effects of anti-bipolar drugs on this pathway. Employing genetic and molecular approaches to identify common targets of Li and VPA in *S. cerevisiae*, we have shown that both Li and VPA affect the inositol metabolic pathway. Both drugs cause a decrease in intracellular myo-inositol and an increase in expression of INO1 as well as the regulatory gene *INO2* required for inositol biosynthesis (20). In addition, both drugs lead to a decrease in the phosphatidylinositol/phosphatidylcholine ratio (38). Furthermore, VPA abrogates the normal response to inositol depletion of inositol-responsive genes and leads to aberrant synthesis of phospholipids (39).

Given the essential role of inositol in cellular function, surprisingly little is known about the regulation of MIP synthase in human cells. Despite some studies on the biochemistry of the enzyme from various animal systems (40–43), the INO1 gene has not been cloned from these sources. Recently, Guan et al. (29) reported the molecular cloning of the putative human MIP synthase cDNA. However, functional expression of the cDNA and characterization of the enzyme was not carried out.

In this study, we cloned the human MIP synthase cDNA and characterized the recombinant enzyme. We show for the first time that the human enzyme is functional in yeast. Furthermore, human MIP synthase activity was decreased in cells grown in the presence of VPA. Because inositol metabolism is thought to play a key role in the etiology and treatment of bipolar illness, conservation of function of this key enzyme underscores the power of the yeast model in studies of this illness.

**EXPERIMENTAL PROCEDURES**

**Materials**—All chemicals used were reagent grade or better. Glucose, yeast extract, and peptone were purchased from Difco. Amino acids, NAD<sup>+</sup>, myo-inositol, glucose 6-phosphate, imidazole, lysozyme, valproate, ampicillin, and chloramphenicol were obtained from Sigma. [14C]Glucose 6-phosphate, [14C]inositol, and [32P]UTP were bought from PerkinElmer Life Sciences. Protease inhibitor tablets were from Roche Applied Science. pRSETA vector, anti-Xpress antibody, and Ni<sup>2+</sup>-chelated resin were purchased from Invitrogen. Dialysis tubing was made by Spectrum Laboratories Inc.

**Strains, Media, and Growth Conditions**—The *S. cerevisiae* strains used in this study are iso1A (derivative of BY4741, his3–1 leu2–0 met15–0 ura3–0 ino1–13, lys2<sup>−</sup>) and wild type BY4741 (isogenic except for INO1), and the indicator strain AID-1 (a/a, ade1Δ his3–1 leu2–0 met15–0 ura3–0 ino1–13 lys2<sup>−</sup>). Yeast strains were grown at 30°C. Synthetic medium contained glucose (2 w/v%), necessary supplements adenine (20 mg liter<sup>−1</sup>), arginine (20 mg liter<sup>−1</sup>), lysine (20 mg liter<sup>−1</sup>), methionine (20 mg liter<sup>−1</sup>), threonine (300 mg liter<sup>−1</sup>), histidine (10 mg liter<sup>−1</sup>), leucine (60 mg liter<sup>−1</sup>), tryptophan (20 mg liter<sup>−1</sup>), and uracil (40 mg liter<sup>−1</sup>), and the salts and vitamin components of Difco vitamin-free yeast base, plus agar (2% w/v) for plates. Complex medium (YPD plates) contained glucose (2% w/v), bactopeptone (2% w/v), yeast extract (1% w/v), and agar (2% w/v). The Escherichia coli strain used is BL21 (DE3) pLysS (Cam/R). E. coli cells were grown at 37°C. LB medium contained tryptone (1% w/v), yeast extract (0.5% w/v), and NaCl (1% w/v). Plates contained 1.5% agar.

**Isolation of Human MIP Synthase cDNA**—A post-mortem human prefrontal cortex specimen derived from a brain collection described previously (44) was used for mRNA purification. mRNA was purified using the Quick-Prep micro mRNA purification kit (Amersham Biosciences) according to the manual. Amplification of the human MIP synthase cDNA was performed as follows. First strand cDNA synthesis was carried out using the first strand cDNA synthesis kit (Amersham Biosciences). Three microliters of the first strand cDNA synthesis product was used for PCR amplification using the primers. PCR was carried out using the following conditions: 25 s at 94°C, 30 s at 55°C, and 1 min at 72°C for 30 cycles. The amplicon was purified using the Wizard PCR Prep Purification System (Promega) and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies). Sequencing reactions were purified using the Wizard PCR Prep Purification System (Promega) and analyzed on an ABI 3730xl genetic analyzer (Life Technologies).

**Cloning and Characterization of Human MIP Synthase**

| Human | --------------- | -------------- | -------------- | -------------- | -------------- | -------------- |
|-------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| S. cerevisiae | ----RTAR--------- | ----RTAR--------- | ----RTAR--------- | ----RTAR--------- | ----RTAR--------- | ----RTAR--------- |

![](image1.png)
Cloning and Characterization of Human MIP Synthase

**Cloning and Characterization of Human MIP Synthase**

The expression of *hINO1* functionally complements yeast *ino1Δ*. A growth of the *ino1Δ* mutant harboring the human MIP synthase cDNA (*ino1Δ/hINO1*) or empty vector (*ino1Δ/vector*) on URA+ and I− medium. B, cells of *ino1Δ/hINO1* were streaked on synthetic I+ medium containing 1 mg/ml 5’-fluoro-orotic acid to select for loss of the plasmid. These cells were then plated (along with controls bottom four vector) on fresh LB medium containing 1 mg/ml 5’-fluoro-orotic acid to select for loss of the plasmid.

**Measurement of Intracellular Inositol**—Intracellular inositol was measured as described (39). Briefly, cells were washed three times and resuspended in water (3 ml/g cells), and glass beads were added to ~50% of the volume of the suspension. Each sample was vortexed for 10 min at 2-min intervals alternating with 2-min incubations on ice. The cell extracts were clarified by centrifugation for 2 min at 2,000 × g, and the supernatants were transferred to Eppendorf tubes and centrifuged for 15 min at 14,000 × g. The supernatants were collected and frozen at −80°C. Intracellular inositol mass per 100 μg of protein was determined by the enzyme-coupled fluorescence assay (45).

**Measurement of *hINO1* Expression**—*ino1Δ/hINO1* cells (the *ino1Δ* mutant transformed with the *hINO1* gene on pRS416GFP) were grown in Ura− synthetic medium in the presence or absence of 0.6 mM VPA to the early stationary phase of growth. Northern analysis was performed as described (39). RNA probes for Northern analysis were synthesized from plasmids linearized with restriction enzymes as follows. The plasmid, restriction enzyme, and RNA polymerase for *hINO1* were pEGM-hINO1, StuI, and Sp6, respectively, and for TCM1 were pAB309, EcoRI, and Sp6, respectively.

**Overexpression of Human MIP Synthase in *E. coli***—The recombinant constructs were transformed into *E. coli* BL21(DE3)pLysS for expression of the protein. A single colony of BL21(DE3)pLysS containing the recombinant pRS416GFP-hINO1 was inoculated into 5 ml of LB medium containing 100 μg/ml ampicillin and 35 μg/ml chloramphenicol. The culture was incubated overnight and used to inoculate 200 ml of LB medium with the same concentration of ampicillin and chloramphenicol. Cells were grown at 37°C to an *A* of 0.4. The recombinant protein was induced by the addition of isopropyl-1-thio-β-D-galactopyranoside to a final concentration of 1 mM followed by incubation for 3 h. Cells were harvested by centrifugation and stored at −80°C until needed.

**Purification of Recombinant Human MIP Synthase**—All the purification steps were carried out at 4°C. Frozen cells (10 g from 8 liters of culture) were thawed and resuspended in 150 ml of binding buffer (50 mM NaPO4, 0.5 M NaCl, 10 mM imidazole, 0.1× protease inhibitor, pH 8.0). The cell suspension was incubated with 100 mg of lysozyme on ice for 30 min and then lysed by sonication 10 times for 30 s on ice. DNase (5 μg/ml) and RNase (10 μg/ml) were added, and this was followed by incubation on ice for another 15 min. The supernatant was separated from cell debris by centrifugation (10,000 rpm for 15 min, SS34 rotor), loaded onto a Ni2+ column (Invitrogen), and allowed to bind with resin for 60 min using gentle agitation to keep the resin suspended. The purification column was then washed five times with 200 ml of washing buffer (50 mM NaPO4, 0.5 M NaCl, 20 mM imidazole, pH 7.0) and eluted with 60 ml of eluting buffer (50 mM NaPO4, 0.5 M NaCl, 250 mM imidazole, pH 8.0). One-ml fractions were collected and analyzed by SDS-PAGE. The peak fractions containing MIP synthase were combined and loaded onto another Ni2+ column. The column was washed and the enzyme was eluted with imidazole.

**Dialysis**—Spectrum Spectra/Por molecularporous membrane dialysis tubing (MWCO 50 kDa, Rancho Dominguez, CA) was prepared according to the suggested protocol. 10-cm strips were cut and soaked in sterile distilled H2O for 10 min. The tubing was filled to 60% of capacity, leaving a small air pocket at one end of the tube. Both ends of the tube were clamped, and the tube was soaked in 4 liters of precooled buffer (1 mM Tris acetate, pH 8.0, 0.05 mM diethiothreitol, 0.02% protease inhibitor). Dialysis was carried out for 38 h at 4°C with the buffer changed every 12 h.

**Protein Assay**—Protein concentration was determined by the method of Bradford (46) using bovine serum albumin as the standard.

**Electrophoresis and Western Blot**—SDS-PAGE was performed on Ready Gel (4–20% gradient) from Bio-Rad. Electrophoresis was carried out using the procedure described by Laemmli (47). All gels were stained with Coomassie Brilliant Blue R-250 (Bio-Rad). Western blots were performed by standard procedures using a mouse-derived monoclonal antibody against Xpress (In vitrogen) for protein expressed in *E. coli*, and alkaline phosphatase goat anti-mouse IgG (Promega) as a secondary antibody.

**MIP Synthase Assay**—MIP synthase was assayed in crude extracts by the chromogenic method of Chen and Charalampous (48). Purified MIP synthase activity was determined by the rapid colorimetric method of Barnett et al. (49) with minor modification. Purified protein was incubated in the reaction buffer in a final volume of 150 μl (100 mM and XhoI and inserted into pRS416GFP and pRSETA vectors. All constructs were confirmed by sequencing.
Tris acetate, pH 8.0, 5 mM G-6-P, 0.8 mM NAD, 2 mM dithiothreitol, if not otherwise indicated) for 1 h at 37°C. 2 mM NH₄Cl was included in the assays for determining optimal pH, temperature, and Kₘ. The reaction was terminated by the addition of 50 µl of 20% (w/v) trichlo-
the supernatant to remove the excess NaIO₄. For the measurement of enzyme activity was measured at the indicated pH values with 100 mM phosphate, a 600-μl reagent mixture (240 μl of H₂O, 120 μl of 2.5% ammonium molybdate, 120 μl of 10% ascorbic acid, and 120 μl of 6 N sulfuric acid) was added and incubated for 1 h at 37 °C. The absorbance was measured at 820 nm, and specific activity was defined as the average of two independent experiments.

Acetic acid and kept on ice for 10 min. The precipitated protein was removed by centrifugation. 200 μl of the supernatant was incubated with 200 μl of NaIO₄ for 1 h. 200 μl of 1 M Na₂SO₃ then was added to the supernatant to remove the excess NaIO₄. For the measurement of phosphate, a 600-μl reagent mixture (240 μl of H₂O, 120 μl of 2.5% ammonium molybdate, 120 μl of 10% ascorbic acid, and 120 μl of 6 N sulfuric acid) was added and incubated for 1 h at 37 °C. The absorbance was measured at 820 nm, and specific activity was defined as units per mg of protein where 1 unit is the amount of enzyme catalyzing the formation of 1 nmol of product per min at 37 °C. For each assay, a second aliquot of the sample was measured for phosphatase activity not released by periodate to control for phosphatase activity. This value was subtracted from the experimental sample to obtain synthase activity.

RESULTS

Isolation of the Human MIP Synthase cDNA

A full-length human MIP synthase cDNA (1677 bp) was isolated from post-mortem brain as described under “Experimental Procedures.” The cDNA encodes a protein of 558 amino acids with a molecular mass of ∼62 kDa. The deduced protein is 50% identical and 69% similar to MIP synthase from S. cerevisiae (Fig. 1).

Interestingly, intracellular inositol in ino1Δ/INO1 transformants was 30% decreased markedly in ino1Δ cells lacking hINO1 but remained constant in cells containing the human gene. Consistent with these data, ino1Δ/hINO1 cells continued to grow, but ino1Δ/vector cells lost viability after 24 h (Fig. 3B). MIP synthase activity was high in ino1Δ cells containing hINO1 but undetectable in ino1Δ cells transformed with empty vector (Fig. 3C). Interestingly, intracellular inositol in ino1Δ/hINO1 was 30%

Functional Expression of Human MIP Synthase cDNA

A yeast-based functional assay was employed to determine whether the cDNA encoded a functional MIP synthase. To perform the assay, hINO1 was cloned into the yeast expression vector pRS426GPD, which is constitutively expressed in high copy (50). This vector was transformed into the yeast ino1Δ mutant, which cannot synthesize inositol because of the disrupted INO1 gene. As shown in Fig. 2A, the ino1Δ mutant transformed with hINO1 (ino1Δ/hINO1) grew on a plate lacking inositol (I̅), in contrast to mutant cells transformed with empty vector (ino1Δ/vector). To confirm that complementation of the yeast ino1Δ mutant was caused by the recombinant construct, 5'-fluoro-orotic acid was used to select for the loss of the plasmid. As shown in Fig. 2B, the ino1Δ cells containing the construct before 5'-fluoro-orotic acid selection grew on I̅-plates, whereas cells that lost the plasmid after 5'-fluoro-orotic acid selection did not. Yeast cells that overexpress the yeast INO1 gene excrete inositol into the medium, creating a phenotype known as opi (overproduction of inositol), which is detected by the ability of the excreted inositol to support growth of an inositol-requiring indicator strain (51). As shown in Fig. 2C, the ino1Δ mutant harboring the hINO1 gene exhibited the opi phenotype. These results indicate that human MIP synthase is functional in yeast.

To further characterize the function of hINO1 in yeast, cells were shifted from I+ to I− medium, and intracellular inositol, MIP synthase activity, and cell viability were assayed. As shown in Fig. 3A, following the shift to I−, intracellular inositol decreased markedly in ino1Δ cells lacking hINO1 but remained constant in cells containing the human gene. Consistent with these data, ino1Δ/hINO1 cells continued to grow, but ino1Δ/vector cells lost viability after 24 h (Fig. 3B). MIP synthase activity was high in ino1Δ cells containing hINO1 but undetectable in ino1Δ cells transformed with empty vector (Fig. 3C). Interestingly, intracellular inositol in ino1Δ/hINO1 was 30%
higher than in isogenic wild type cells lacking the hINO1 gene (Fig. 3D), which most likely accounts for the inositol excretion phenotype of ino1ΔhINO1 observed in Fig. 2C. This can be explained by the constant high level of expression of the hINO1-containing plasmid. In contrast, expression of the native yeast INO1 gene in wild type cells is regulated and expressed highly only in logarithmic phase cells (32).

Effect of VPA on Inositol and MIP Synthase Activity

We have shown that both Li and VPA cause a decrease in intracellular inositol in yeast (20). This is consistent with the observed decrease in brain inositol levels following Li (52) and VPA (23) treatment. The Li-induced decrease can be explained by inhibition of inositol monophosphatase (19, 21). However, a decrease in MIP levels was observed during growth in the presence of VPA, suggesting that VPA may inhibit MIP synthase (20). To address this possibility, inositol and MIP synthase activities were assayed in crude extracts of ino1ΔhINO1 grown in the presence of 0.6 mM VPA (the concentration used in therapeutical treatment). As shown in Fig. 4, in the presence of VPA, intracellular inositol was decreased 25% (Fig. 4A), and a 35% decrease in MIP synthase activity was observed (Fig. 4B). The gene was expressed at high levels in all growth phases (data not shown), and expression was not affected by VPA (Fig. 4C), indicating that the inhibitory effect of the drug was at the level of the protein.

Purification of Recombinant Human MIP Synthase

Human MIP synthase was expressed following isopropyl-1-thio-β-D-galactopyranoside induction of E. coli BL21(DE3)-pLysS transformed with hINO1 (Fig. 5A, lanes 2–5). The His-tagged human MIP synthase protein was purified by Ni²⁺ affinity chromatography and eluted with 250 mM imidazole.

The purified protein analyzed by SDS-PAGE migrated with an apparent molecular mass of ~65 kDa (Fig. 5A, lane 6) which is consistent with the expected size (62-kDa protein plus a 3-kDa tag). Western blot analysis using anti-Xpress antibody confirmed that this was the recombinant protein (Fig. 5B, lanes 2–6). The specific activity of the purified enzyme was measured by the rapid colorimetric method of Barnett et al. (49) and confirmed by the chromatographic assay of Chen and Charalampous (48) using [14C]glucose 6-phosphate as a substrate.

Properties of Human MIP Synthase

Effect of pH and Temperature on Enzyme Activity—Human MIP synthase activity was measured at pH 6.0–10.0 using 100 mM Tris acetate buffer. All other components in the reaction mixture were kept constant, and assays were performed using standard conditions as described under “Experimental Procedures.” The optimal pH of human MIP synthase was 8.0 (Fig. 6A).

Enzyme activity was measured in a controlled temperature water bath from 20 to 60 °C under standard assay conditions. The temperature profile suggested that maximum activity was obtained at 37 °C, and the enzyme still was 90% active at 45 °C (Fig. 6B). Thermal stability of the enzyme was determined by assaying samples heated for 20 min at 30–60 °C. The enzyme was stable to heating up to 45 °C. However, ~60% activity was lost upon heating at 60 °C (Fig. 6C).

Enzyme Kinetics—Kinetic analysis of purified recombinant enzyme is shown in Fig. 7. When NAD⁺ was held constant at 0.8 mM and the G-6-P concentration varied, saturation kinetics were shown by the enzyme. The apparent $K_m$ for G-6-P was 0.57 mM (Fig. 7A). Saturation kinetics also were shown when G-6-P was held constant at 5 mM and the NAD⁺ concentration varied. The apparent $K_m$ for NAD⁺ was 8 μM (Fig. 7B).

Effect of Cations on Enzyme Activity—MIP synthase activity was determined in the presence of NH₄⁺, Na⁺, K⁺, Mg²⁺, Mn²⁺, Ca²⁺, and Zn²⁺. Like MIP synthase from all other organisms except Archaeoglobus fulgidus (2), human MIP synthase was strongly stimulated by NH₄⁺. The activity increased 5-fold at 5 mM NH₄⁺ (Fig. 8A). 10 mM K⁺ stimulated activity 5-fold. Mg²⁺ and Mn²⁺ increased activity 2–3-fold at 1 or 10 mM (Fig. 8B). Activity was decreased 5-fold in the presence of 1 mM Zn²⁺ and was totally inactivated in the presence of 10 mM Zn²⁺ (Fig. 8B). Na⁺ and Ca²⁺ had no effect on the enzyme at 1 mM and increased activity by about 2-fold at 10 mM (Fig. 8B).

Effect of VPA on Purified Human MIP Synthase—To determine whether the decrease in MIP synthase activity observed during growth in the presence of VPA (Fig. 4B) is caused by direct inhibition of the enzyme, purified human MIP synthase activity was determined in the presence of different concentrations of the drug. The enzyme was not inhibited by the therapeutic concentration of VPA and was only slightly (10%) inhibi-
it by VPA at 10 mM (Fig. 9). This inhibition was not seen when 14 mM NH$_4^+$ was included in the reaction mixture (data not shown).

**DISCUSSION**

We report for the first time that human MIP synthase is functional in yeast and supports growth of the yeast ino1 mutant in the absence of inositol. In the presence of VPA leads to decreased inositol monophosphate and intracellular inositol and increased INO1 expression consistent with the inhibition of yeast MIP synthase (20). Similar to the observations in yeast cells, a decrease in MIP synthase activity also was observed in crude extracts from post-mortem human brain.

The purified recombinant human enzyme was not inhibited by therapeutic concentrations of VPA (Fig. 9). Purified yeast MIP synthase was similarly not affected by VPA. These findings suggest that a metabolite of VPA or an inhibitor that accumulates in the presence of VPA may be responsible for MIP synthase inhibition. The current findings support the use of the yeast model to elucidate the effects of VPA on inositol biosynthesis.

The properties of the recombinant MIP synthase were similar mostly to those reported for native enzymes. The enzyme was active from pH 7.5 to 10.0. MIP synthase that was partially purified had a pH optimum of 7.5 (53). The $K_m$ for the recombinant enzyme for NAD$^+$ was 8 $\mu$m, much lower than 452 $\mu$m reported for the partially purified human fetal brain enzyme but consistent with values from other sources, e.g. 11 $\mu$m for the bovine testis enzyme (42), 17.9 $\mu$m for the rat testis enzyme (40), and 8 $\mu$m for the yeast enzyme (27). NH$_4^+$ strongly stimulated both the partially purified fetal brain enzyme and the recombinant enzyme. Other mono- and divalent cations also stimulated the enzyme to different extents, which may implicate a general ionic effect. The mono- and divalent cations also stimulated the enzyme to different extents, which may implicate a general ionic effect. The enzyme was active from pH 7.5 to 10.0. MIP synthase that was partially purified had a pH optimum of 7.5 (53). The $K_m$ for the recombinant enzyme for NAD$^+$ was 8 $\mu$m, much lower than 452 $\mu$m reported for the partially purified human fetal brain enzyme but consistent with values from other sources, e.g. 11 $\mu$m for the bovine testis enzyme (42), 17.9 $\mu$m for the rat testis enzyme (40), and 8 $\mu$m for the yeast enzyme (27). NH$_4^+$ strongly stimulated both the partially purified fetal brain enzyme and the recombinant enzyme. Other mono- and divalent cations also stimulated the enzyme to different extents, which may implicate a general ionic effect.

Although the therapeutic mechanism of action of Li and VPA are not known, both anti-bipolar drugs cause a decrease in intracellular inositol. In yeast, inositol is a major metabolic invariant. Inositol is a major metabolic invariant. Inositol is a major metabolic invariant. Inositol is a major metabolic invariant. Inositol is a major metabolic invariant. Inositol is a major metabolic invariant. Inositol is a major metabolic invariant. Inositol is a major metabolic invariant. Inositol is a major metabolic invariant. Inositol is a major metabolic invariant. Inositol is a major metabolic invariant. Inositol is a major metabolic invariant. Inositol is a major metabolic invariant. Inositol is a major metabolic invariant. Inositol is a major metabolic invariant. Inositol is a major metabolic invariant. Inositol is a major metabolic invariant. Inositol is a major metabolic invariant. Inositol is a major metabolic invariant. Inositol is a major metabolic invariant. 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3 D. Ding and M. L. Greenberg, unpublished observations.