Communications to the Editor

Inhibition of *myo*-Inositol-1-phosphate Synthase by a Reaction Coordinate Intermediate

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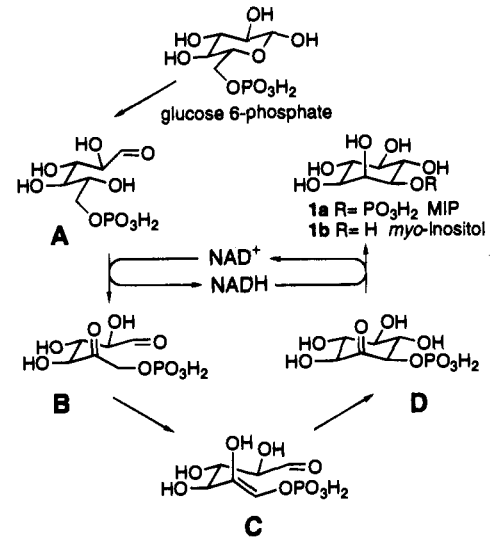
In vivo concentrations of *myo*-inositol in the human brain play a critical role as a secondary messenger in neurochemistry and as a hypothesized determinant of manic depression.[1] Conversion of D-glucose 6-phosphate into *myo*-inositol 1-phosphate (MIP) catalyzed by the enzyme MIP synthase (EC 5.5.1.4) is the first step in de novo biosynthesis of *myo*-inositol (Scheme 1) in brain tissue that is otherwise segregated from dietary *myo*-inositol.[2] Inhibition of MIP synthase and resultant reductions in *myo*-inositol levels might thus be an alternative to lithium for treatment of manic depression. En route to this goal, a proposed reaction coordinate intermediate, *myo*-2-inosose 1-phosphate (**D**, Scheme 1), has been synthesized and discovered to be a potent competitive inhibitor of MIP synthase.

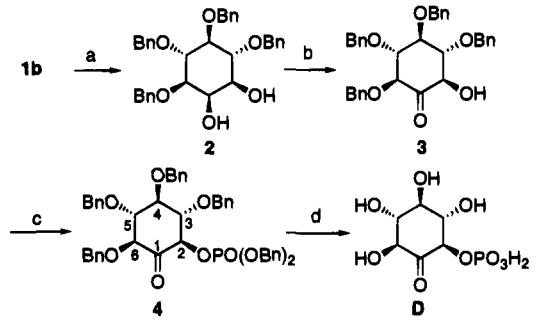
MIP synthase catalyzes (Scheme 1) an alcohol oxidation, enolization, intramolecular aldol condensation, and carbonyl reduction during each turnover of substrate into product. The reduced β-nicotinamide adenine dinucleotide (NADH) generated during formation of intermediate **B** is reoxidized to NAD when intermediate **D** (*myo*-2-inosose 1-phosphate) is reduced to product MIP. This absence of any net consumption of NAD categorizes MIP synthase in a unique subgroup of NAD-requiring enzymes which exploit NAD as a catalyst rather than a cosubstrate.[3] As potential enzyme inhibitors, stable analogues of reactive intermediates[4] have typically been the focus of synthetic and enzymological attention given the reactivity and relative instability of most reaction coordinate intermediates. By contrast, intermediate *myo*-2-inosose 1-phosphate (**D**, Scheme 1) appeared to be a molecule possessing sufficient stability to allow its synthesis (Scheme 2) and characterization. The existence of *myo*-2-inosose 1-phosphate, prior to this work, was inferred by radiolabeling experiments.[5] Previous attempts to synthesize this intermediate have been unsuccessful.[5b]

A series of protection and deprotection steps were employed to obtain intermediate diol **2** from *myo*-inositol.[6] Selective oxidation[7] of the diol using dibutyltin oxide and bromine in the presence of tributyltin methoxide yielded a 3:1 regioisomeric product mixture, from which ketone **3** was purified by crystallization. The structure of ketone **3** was confirmed by hydrogenation and comparison with commercially available *myo*-2-inosose. Subsequent reaction of ketone **3** with sodium hydride

Scheme 1



Scheme 2



[a] (a) (i) $(MeO)_2C(CH_3)_2$, *p*-TsOH, DMSO, 110 °C, 83%, (ii) NaOH, BnBr, 120 °C, (iii) HCl (6 N), MeOH, reflux, 58%; (b) (i) Bu_2SnO, MeOH, reflux, (ii) Br_2, CH_2Cl_2, Bu_3SnOMe, 50%; (c) (i) NaH, THF, 0 °C, (ii) TBPP, THF, 0 °C to room temperature, 61%; (d) H_2, 10% Pd on C, H_2O/THF (1:6), 99%.

and tetrabenzyl pyrophosphate (TBPP) afforded benzylated phosphoroketone **4** and an α,β-unsaturated phosphoroketone resulting from elimination of the C-3 benzyl alcohol (Scheme 2) of **4**. Although the elimination side product could be minimized by use of NaH that was free of NaOH contamination, chromatography followed by crystallization was required to obtain pure **4**. All of the benzyl protecting groups were then removed in a single step by catalytic hydrogenation to afford racemic intermediate **D** in 15% overall yield.

The nature of the interaction between the proposed reaction coordinate intermediate, *myo*-2-inosose 1-phosphate, and MIP synthase depended on the redox state of the enzyme active site. MIP synthase employed in these experiments was purified to homogeneity from *Saccharomyces cerevisiae* MW 5.55.[8] Treatment of the enzyme with activated charcoal afforded *apo*MIP synthase, which was reconstituted with NADH. Incubation of *myo*-2-inosose 1-phosphate with the NADH–MIP synthase complex resulted (Figure 1) in a decrease in absorption at 340 nm corresponding to the oxidation of NADH. Formation of *myo*-inositol 1-phosphate from *myo*-2-inosose 1-phosphate was confirmed by FAB mass spectrometry. Although *myo*-2-inosose

(1) (a) Sherman, W. R.; Leavitt, A. L.; Honchar, M. P.; Hallcher, L. M.; Phillips, B. E. *J. Neurochem.* **1981**, *36*, 1947. (b) Drummond, A. H. *Trends Pharmacol. Sci.* **1987**, *8*, 129.

(2) (a) Margolis, R. U.; Press, R.; Altszuler, N.; Stewart, M. A. *Brain Res.* **1971**, *28*, 535. (b) Sherman, W. R. In *Inositol Lipids in Cell Signalling*; Michell, R. H., Drummond, A. H., Downes, C. P., Eds.; Academic: New York, 1989; p 39.

(3) Frey, P. A. In *Pyridine Nucleotide Coenzymes Chemical, Biochemical, and Medical Aspects Part B*; Dolphin, D., Poulson, R., Avromovic, O., Eds.; Wiley: New York, 1987; p 461.

(4) (a) Schloss, J. V. *Acc. Chem. Res.* **1988**, *21*, 348. (b) Morrison, J. F.; Walsh, C. T. *Adv. Enzymol.* **1988**, *61*, 201.

(5) (a) Chen, C. H.-J.; Eisenberg, F., Jr. *J. Biol. Chem.* **1975**, *250*, 2963. (b) Byun, S. M.; Jenness, R. *Biochemistry* **1981**, *20*, 5174.

(6) Gigg, R.; Warren, C. D. *J. Chem. Soc. C* **1969**, 2367.

(7) David, S.; Hanessian, S. *Tetrahedron* **1985**, *41*, 643.

(8) The purification employed was a modification of a published protocol: Donahue, T. F.; Henry, S. A. *J. Biol. Chem.* **1981**, *256*, 7077.

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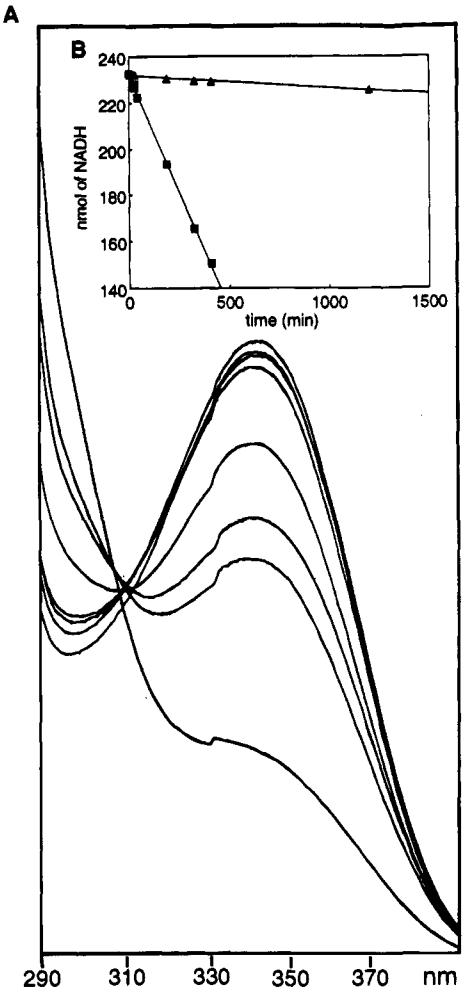

Figure 1. Incubation of *myo*-2-inosose 1-phosphate with the complex of NADH and *apo*MIP synthase: (A) The UV−vis absorbance profiles of the reaction solution at 0, 27, 27.5, 44, 186, 325, 408, and 1200 min when scanned from 250 to 400 nm; (B) the change in absorbance of the reaction solution when monitored at 340 nm with time (■); overlapping progess lines for control experiments where *myo*-2-inosose 1-phosphate was incubated with NADH in a solution lacking *apo*MIP synthase or in a separate experiment, where NADH was incubated with *apo*MIP synthase in a solution lacking *myo*-2-inosose 1-phosphate (▲).

1-phosphate was a substrate for *apo*MIP synthase reconstituted with NADH, this same molecule was a competitive inhibitor of native MIP synthase possessing bound NAD. The observed inhibition constant[9] (K_i) of 3.6×10^{-6} M contrasts with the Michaelis constant (K_m) of 1.2×10^{-3} M for substrate D-glucose 6-phosphate.

Potent enzyme inhibition by reactive intermediate analogues can be interpreted as arising from strong stabilizing interactions between functional groups possessed by the analogue and active site residues normally responsible for binding of the reactive intermediate.[4] Inhibition of MIP synthase by *myo*-2-inosose 1-phosphate might reflect such stabilizing interactions between enzyme and one of its reactive intermediates. However, the complex of MIP synthase, NAD, and intermediate **D** is not a naturally-occurring configuration. When MIP synthase binds intermediate **D** during catalyzed turnover of D-glucose 6-phosphate into *myo*-inositol 1-phosphate, NADH is present at the active site. Oxidation of NADH and concomitant reduction of intermediate **D** may even drive an essential enzyme conformational change. Viewed from this perspective, MIP synthase inhibition is not necessarily a consequence of stabilizing interactions between the active site and *myo*-2-inosose 1-phosphate. Inhibition may instead result from a kinetic barrier to release of *myo*-2-inosose 1-phosphate caused by the absence of an active site reductant when *myo*-2-inosose 1-phosphate is bound by MIP synthase.

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Supplementary Material Available: Experimental procedures for enzymological manipulations along with synthesis and characterization of Scheme 2 intermediates **2**−**4** and product *myo*-2-inosose 1-phosphate (intermediate **D**) (4 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(9) This constant has not been adjusted to reflect the likely inhibition of MIP synthase by only one enantiomer of the synthesized racemic mixture. Whether the enantiomer of intermediate **D** present in the synthesized racemic mixture might also inhibit MIP synthase is a possibility that remains to be explored.

(10) Barnett, J. E. G.; Brice, R. E.; Corina, D. L. *Biochem J.* **1970**, *119*, 183.