Communications to the Editor

myo-Inositol 1-Phosphate Synthase: Does a Single Active-Site Amino Acid Catalyze Multiple Proton Transfers?

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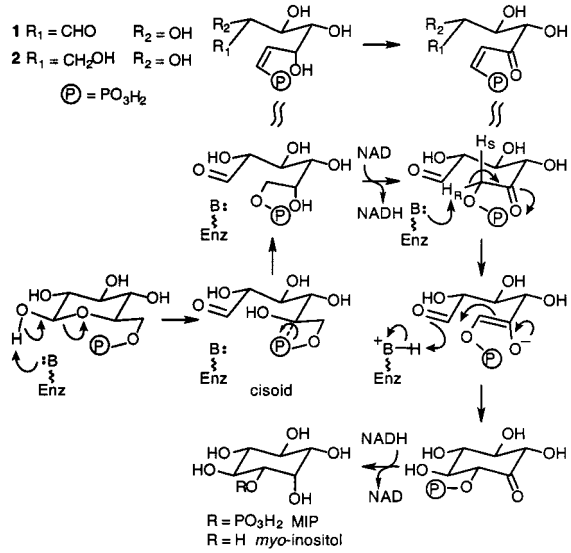
In eucaryotes, inositol and its various phosphate monoesters play essential roles in both cellular structure and regulation of cellular processes.[1] *myo*-Inositol 1-phosphate (MIP) synthase catalyzes the first step in inositol biosynthesis, involving conversion of D-glucose 6-phosphate into *myo*-inositol 1-phosphate. As opposed to most NAD-requiring enzymes that use NAD as a cosubstrate, MIP synthase employs (Scheme 1) its NAD as an active-site catalytic residue.[2] Floss has proposed that a single, active-site base and its conjugate acid mediate all of the nonredox steps separating the substrate from the product.[3] To test this mechanistic hypothesis, conformationally restricted (*Z*)- and (*E*)-vinylhomophosphonate analogues of the substrate D-glucose 6-phosphate as well as substrate analogues effectively locked in cyclic, pyranoside forms were synthesized and tested for binding to the active site of MIP synthase. Results suggest that MIP synthase exploits the phosphate monoester of 5-keto-D-glucose 6-phosphate (Scheme 1) to catalyze the intramolecular aldol condensation of this reactive intermediate and that the enzyme selectively binds the acyclic, free carbonyl rather than the cyclic, pyranosyl form of the substrate D-glucose 6-phosphate.

The first step in the Floss mechanism[3] entails MIP synthase binding of the β-anomer[4] of D-glucose 6-phosphate in a cisoid conformation where C-5, C-6, O-6, and P form a plane with the phosphorus atom positioned along the C-4/C-5 axis (Scheme 1). Removal of the proton from the C-1 hydroxyl group by the active-site base followed by delivery of the abstracted proton to O-5 catalyzes ring opening. A 120° rotation around the C-4/C-5 bond then positions C-6 so that the *pro*-6*R* hydrogen atom is selectively removed by the active-site base after oxidation of the C-5 hydroxyl group. Subsequent delivery of the abstracted proton to the C-1 carbonyl oxygen, aldol condensation, and reduction of the C-5 carbonyl complete the catalytic cycle. This exquisite mechanistic proposal presents MIP synthase as a model of catalytic efficiency. The enzyme economizes on the number of required binding pockets and conformational changes since conversion of substrate to product is accomplished in lieu of significant changes in the positions of the phosphate monoester, of the C-1 carbonyl, or of the hydroxyl groups attached to C-2, C-3 and C-4. Overall, the active-site base and its conjugate acid are formulated to mediate four proton transfers.

Scheme 1



Scheme 2

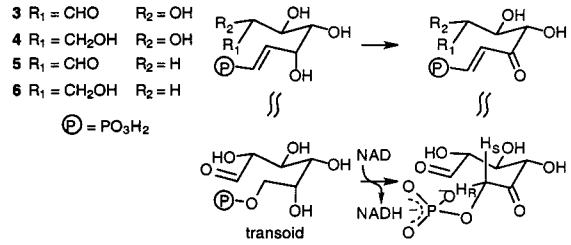


Table 1. MIP Synthase Binding of (*E*)-Vinylhomophosphonate Substrate Analogues

	inhibition type	k_{on} (M^{-1} s^{-1}) k_{off} (s^{-1})	K_i (M)	E-NADH formation
3	competitive		1.1×10^{-3}	+
4	slowly reversible	60 1.8×10^{-3}	30×10^{-6}	+
5	competitive		0.10×10^{-3}	+
6	slowly reversible	4.3×10^{3} 2.9×10^{-3}	0.67×10^{-6}	+

6-Deoxy-D-glucose 6-(*Z*)-vinylhomophosphonate, **1**, and 6-deoxy-D-glucitol 6-(*Z*)-vinylhomophosphonate, **2**, were designed as structural mimics of the enzyme-bound cisoid isomers of D-glucose 6-phosphate formulated by the Floss hypothesis (Scheme 1). However, neither (*Z*)-vinylhomophosphonates **1** or **2** were inhibitors. By contrast (Scheme 2), 6-deoxy-D-glucose 6-(*E*)-vinylhomophosphonate, **3**, and 6-deoxy-D-glucitol 6-(*E*)-vinylhomophosphonate, **4**, were competitive inhibitors (Table 1). Given the observation[5] that removal of the C-2 hydroxyl group improves inhibition of MIP synthase, 2,6-dideoxy-D-glucose 6-(*E*)-vinylhomophosphonate, **5**, and 2,6-dideoxy-D-glucitol 6-(*E*)-vinyl-

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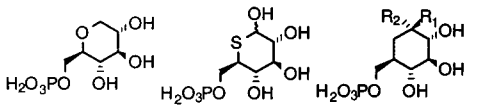
homophosphonate, **6**, were synthesized (Scheme 2). Both **5** and **6** were competitive inhibitors (Table 1).

6-Deoxy-D-glucitol 6-(*E*)-vinylhomophosphonate, **4**, and 2,6-dideoxy-D-glucitol 6-(*E*)-vinylhomophosphonate, **6**, are the first reported slowly reversible inhibitors of MIP synthase (Table 1). 2,6-Dideoxy-D-glucitol 6-(*E*)-vinylhomophosphonate, **6**, is the most potent inhibitor ($K_i = 0.67 \times 10^{-6}$ M) of MIP synthase discovered to date.[5,6] The Michaelis constant (K_m) for the substrate D-glucose 6-phosphate is 1.2×10^{-3} M.[6] Formation of NADH as evidenced by an increase in optical density at 340 nm was also observed during incubation of MIP synthase with (*E*)-vinylhomophosphonates **3**−**6**. Although intermediacy of 5-keto-D-glucose 6-phosphate and NADH during MIP synthase catalysis have been implicated[7] by isotope effects and trapping experiments, oxidation of (*E*)-vinylhomophosphonates **3**−**6** provides the first direct observation of NADH formation.

The absence of detectable active-site interaction with (*Z*)-vinylhomophosphonate **1** and **2** is inconsistent with cisoid conformations (Scheme 1) of D-glucose 6-phosphate and 5-keto-D-glucose 6-phosphate playing a role in MIP synthase catalysis. Competitive inhibition observed for (*E*)-vinylhomophosphonate **3**−**6** is, however, suggestive that transoid conformations (Scheme 2) of D-glucose 6-phosphate and 5-keto-D-glucose 6-phosphate may be found along the reaction coordinate catalyzed by MIP synthase. A transoid conformation would position the dibasic phosphate monoester of 5-keto-D-glucose 6-phosphate for removal of a C-6 methylene proton (Scheme 2). Stereoselective proton removal would follow from the active site's positioning of the phosphate monoester and C-5 carbonyl in addition to a preferred H_R−C(6)−C(5)−O(5) dihedral angle of 90° for removal of the *pro*-6*R* hydrogen atom (Scheme 2). The resulting monobasic phosphate monoester could then deliver the abstracted proton back to the C-1 carbonyl oxygen to complete its catalytic role in the formation of the bond between C-1 and C-6. Intramolecular removal of a proton by a phosphate monoester is precedented[8] for 3-dehydroquinate synthase, an enzyme mechanistically related to MIP synthase in its catalytic employment of NAD. Conformationally restrained substrate analogues were likewise used to implicate intramolecular proton removal by the phosphate monoester of the substrate of 3-dehydroquinate synthase.[8]



Use of a phosphate monoester to catalyze the aldol condensation brings into question the mechanism employed by MIP synthase for opening of the pyranosyl ring of D-glucose 6-phosphate. An important clue in this respect is the observation that 6-deoxy-D-glucitol 6-(*E*)-vinylhomophosphonate, **4**, is a 37-fold more potent inhibitor of MIP synthase than 6-deoxy-D-glucose 6-(*E*)-vinylhomophosphonate, **3**, and that 2,6-dideoxy-D-glucitol 6-(*E*)-vinylhomophosphonate, **6**, is a 149-fold more potent inhibitor relative to 2,6-dideoxy-D-glucose 6-(*E*)-vinylhomophosphonate, **5**. This pronounced difference in active-site binding correlates with glucose analogues **3** and **5** existing primarily as cyclic pyranosides in aqueous solution, whereas glucitols **4** and **6** are acyclic substrate analogues. To further explore the possibility that MIP synthase selectively binds the acyclic form of D-glucose 6-phosphate, 1,5-anhydro-D-glucose 6-phosphate, **7**, 5-thio-D-glucose 6-phosphate, **8**, carbocyclic β-D-glucose 6-phosphate, **9**, and carbocyclic α-D-glucose 6-phosphate, **10** were synthesized. None of these inhibitors that are covalently locked in cyclic form (**7**, **9**, **10**) or that undergo ring opening[9] very slowly (**8**) led to any detectable inhibition of MIP synthase.

The enzyme inhibition observed for (*E*)-vinylhomophosphonates **3**−**6** should provide important clues for the design of future MIP synthase inhibitors. From an enzymology perspective, the active site's configurational preference for binding (*E*)-vinylhomophosphonates **3**−**6** versus (*Z*)-vinylhomophosphonates suggests that MIP synthase recruits the phosphate monoester of substrate D-glucose 6-phosphate as the base that catalyzes intramolecular aldol condensation. The pronounced differences in inhibitory potencies for glucitols **4** and **6** relative to the corresponding glucose analogues **3** and **5** coupled with the complete absence of enzyme inhibition observed for cyclic D-glucose 6-phosphate analogues **7**−**10** are inconsistent with MIP synthase binding the cyclic pyranosyl form of substrate followed by enzyme-catalyzed ring opening. Instead, MIP synthase is apparently content to selectively bind the acyclic, free carbonyl form of its substrate, which constitutes less than 0.4% of D-glucose 6-phosphate present in aqueous solution.[10] A mechanistic picture of MIP synthase thus emerges of an enzyme that may be less a paragon of catalytic efficiency than a paradigm of catalytic opportunism.

Supporting Information Available: Evaluation of MIP synthase inhibition and synthesis of substrate analogues **1**−**10** Schemes 3−9 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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