Structure of *Myo*-Inositol-1-Phosphate

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Introduction

Inositol-containing compounds play critical and diverse biological roles including signal transduction and second messenger signalling, stress response and cell wall biogenesis.[1-4] The biosynthesis of inositol follows a common pathway involving first the conversion of glucose-6-phosphate to 1L-*myo*-inositol-1-phosphate (MIP) catalyzed by MIP synthase, followed by dephosphorylation catalyzed by MIP phosphatase.[3] The synthesis of MIP is both the first committed and rate limiting step in this pathway.[3] Significant biosynthesis of inositol has been detected in organs where a significant blood barrier exists, such as the testes or brain.[5-9] Significantly, reduction of the brain inositol pool by inhibiton of MIP phosphatase has been suggested to be the mode of action for lithium in the treatment of bipolar disorder.[10-13] Recent *in vivo* results in yeast suggest that valproate, a drug used in the treatment of depression, bipolar disorder and seizure disorder may act by inhibition of MIP synthase in this pathway.[14]

Materials and methods

Crystallization of the native MIP synthase was described previously. A three-wavelength MAD data set was collected on beamline 19-ID of the Structural Biology Center (Advanced Photon Source, Argonne, IL), and the data were processed using HKL2000.[15] From the anomalous data sets, the seleno-methionines were found using SOLVE.[16] Of the 22 Se sites expected for the dimer, SOLVE successfully found 18. From these sites, SOLVE also generated an interpretable electron density map. After solvent flattening using the CCP4 package,[17] the map was traced using both the O[18] and TURBO-FRODO packages. The trace was then initially refined using CNS.[19] After multiple refinement rounds, the final model at 2.4 Å has an R and free-R values of 20.5% and 24.3% and contains 553 water molecules. All residues except for 1.1% of the entire structure (6 residues) lie within the most favored or allowed regions of the Ramachandran plot. Similarly, the parameters evaluated by PROCHECK[20] are well within the bounds established from well-refined structures at equivalent resolution.

Discussion

This structure, combined with the recently determined structure of the inhibitor-bound form of MIP synthase, demonstrates an extreme case of induced fit, where fully 60 amino acids become ordered upon binding, resulting in the complete encapsulation of the substrate within the active site. It therefore appears that a significant folding/unfolding transition must occur to allow substrate binding/dissociation. We will be able to use these structures to model the interaction of valproate with the enzyme. Valproate is an important anti-seizure antipsychotic used in the treatment of a host of mental illnesses.

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