Abstract

Inositol monophosphatase (IMPase) is a Mg^{2+} activated and Li^{+} inhibited phosphatase that hydrolyzes phosphomonoester bond of *myo*-inositol-1-phosphate (I-1-P). IMPase plays a crucial role in the development and proper functioning of the brain due to its involvement in phosphoinositol signalling cascade. IMPase is an important target of Li^{+}-based therapeutics for manic depressive disorders. Despite the therapeutic efficiency of Li^{+}, the precise mechanism of the Li^{+}-induced inhibition of IMPase remains obscured. IMPase shows the highest catalytic activity with the substrate I-1-P, but it also manifests substrate promiscuity with other substrates like 2'AMP and β-glycerophosphate. Attempts have been made with staphylococcal IMPase-I (SaIMPase-I; SAS2203) and IMPase-II (SaIMPase-II; SAS1042) to decipher the (i) molecular basis of substrate promiscuity, (ii) mode of inhibition by metal ions like Li^{+} and high concentration of Mg^{2+} and (iii) possible role of SaIMPase-II in staphylococcal biofilm formation.

Crystal structures of SaIMPase-II and its substrate (I-1-P and 2'AMP) bound complexes have revealed the role of active site mobile loops (connecting α1-α2, α4-β6, α6-β8 and α5-β7) in substrate selection. Furthermore, considering the high degree of structural homology and conserved active site residues of SaIMpase-I with human IMPase-I, SaIMPase-I is used as a model enzyme to study molecular details of Li^{+} inhibition. Li^{+} inhibition in IMPase is competitive with Mg^{2+} and shares a common or overlapping binding site. Li^{+} is virtually invisible in X-ray crystallography since Li^{+} has only two electrons to interact with X-ray. The crystal structure of SaIMPase-I ternary product complex (SaIMPase-I/$3Mg^{2+}$/PO_4^{3-}) shows a bound phosphate and three Mg^{2+} (namely Mg1, Mg2 and Mg3) in the active site. The competitive displacement of Mg^{2+} as a function of an increasing LiCl concentration in the crystals of SaIMPase-I ternary product complex has been employed to visualize the binding site of Li^{+}. The complete absence of the electron density corresponding to the pre-occupied Mg2 is observed in LiCl soaked structures. Moreover, based on a detailed investigation of the phosphate conformation and the coordination state of the bound Mg^{2+} ions, the inhibition mechanisms of Li^{+} and high concentrations of Mg^{2+} are proposed. It is also reported that the SaIMPase-II is essential in staphylococcal polysaccharide-independent biofilm formation. Overexpression of SaIMPase-II in *E. coli* produces extracellular macroscopic fibers and it is confirmed that the fibers are composed of SaIMPase-II. The amyloidic nature of the fiber has been evaluated by high-resolution electron microscopy complemented with X-ray fiber diffraction and binding of amyloid-specific dyes, such as Congo red and Thioflavin-T. The fibers are found to be highly sticky in nature and bind a large number of bacterial cells. It is suggested that SaIMPase-II is involved in fibers formation and acts as the adhesion in biofilm matrix.

Key words

Inositol monophosphatase; *Staphylococcus aureus*; crystal structure; substrate specificity; Li^{+} inhibition; amyloid fiber.