Prediction of substrate-binding site and elucidation of catalytic residue of a phytase from Bacillus sp.

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Abstract

The present study primarily deals with the identification of substrate-binding site and elucidation of catalytic residue of the phytase from Bacillus sp. (Genbank Accession No. EF536824) employing molecular modeling and site-directed mutagenesis. Homology-based modeling of the Bacillus phytase revealed α-propeller structure with twelve active-site aminoacid residues, viz., D75, R77, Y78, H138, Q140, D189, D190, E191, Y238, Y239, N346 and R348. Docking of substrate Ins(1,2,3,4,5,6)hexakisphosphate with the phytase model disclosed interaction of Y78 residue with the sixth position phosphate, while D75 and R77 residues revealed hydrogen bonding with the fifth position phosphate of the phytate. Analysis of hydrolysis products of phytate indicated the sequential removal of alternate phosphates, resulting in the formation of final product Ins triphosphate. Mutant phytases Y78A/F, derived from site-directed mutagenesis, exhibited complete loss of enzyme activity despite substrate binding, thereby suggesting the intrinsic role of Y78 residue in the catalytic activity. The Bacillus mutant phytases can be used to generate enzyme crystals complexed with phytate and lower Ins phosphates for indepth analysis of substrate binding and catalytic activity of the enzyme.

References


