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Detecting the Structural Determinants of Glycosyl Hydrolase Family 11 Xylanase Inhibition

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Microbial (endo)xylanases (endo-β-1,4-xylanases, E.C.3.2.1.8) are frequently used to modify the functionality of (arabino)xylan in feed and food applications. In cereals two types of endogeneous proteinaceous xylanase inhibitors were discovered, *i.e.* TAXI-type (*T. aestivum* xylanase inhibitor) [1] and XIP-type (xylanase inhibiting protein) [2] inhibitors.

Probing the inhibition activities for different isolated TAXI-isoforms against both *A. niger* and *B. subtilis* xylanases, led to the definition of TAXI-I type, with inhibiting activity against both GH family 11 endox, and TAXI-II type, with inhibition specificity against the *B. subtilis* endoxylanase only [3].

Studies on the molecular identification and characterization of the TAXI-encoding genes showed that TAXIs are a new class of plant proteins to which a function as plant protective microbial glycoside hydrolase inhibitor can be ascribed [4].

Both structures of TAXI-I in complex with *A. niger* xylanase and in complex with *B. subtilis* xylanase were determined. Detailed comparison of both complexes reveals the xylanase and inhibitor amino acid residues that are critical for complex-binding and stabilization. Extrapolation towards TAXI-II type inhibitors shows that TAXI-I inhibition activity is independent of the pH optima of xylanases, while TAXI-II inhibition of xylanases with low pH optima will be weak or absent [5].

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Keywords: protein-inhibitor binding, glycosyl hydrolases, plant biotechnology

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The Mechanism of *T. cruzi* FPPS Involves a Substrate Induced Conformational Change

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Typanosoma cruzi, the causative agent of Chagas disease, has recently been shown to be sensitive to the action of the bisphosphonates currently used in bone resorption therapy. These compounds target the mevalonate pathway by inhibiting farnesyl diphosphate synthase (farnesyl pyrophosphate synthase, FPPS), the enzyme that condenses the diphosphates of C_5 alcohols (isopentenyl and dimethylallyl) to form C_{10} and C_{15} diphosphates (geranyl and farnesyl). The structures of the *T. cruzi* FPPS (*Tc*FPPS) alone and in two complexes with substrates and inhibitors reveal that following binding of the two substrates and three Mg^{2+} ions, the enzyme undergoes a major conformational change consisting of a hinge-like closure of the binding site. In this conformation, it would be possible for the enzyme to bind a bisphosphonate inhibitor that spans the sites usually occupied by dimethylallyl diphosphate (DMAPP) and the homoallyl moiety of isopentenyl diphosphate. In addition, the structures provide an important mechanistic insight: after its formation, geranyl diphosphate can swing without leaving the enzyme, from the product site to the substrate site to participate in the synthesis of farnesyl diphosphate.

Keywords: enzyme mechanism, inhibitor design, farnesyl diphosphate synthase

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Crystallographic Analysis Identifies a Novel Enzymatic Reaction

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A novel enzymatic reaction has been identified with the help of crystallographic analysis. *In vivo*, orotidine 5'-monophosphate decarboxylase (ODCase) catalyzes the conversion of orotidine 5'-monophosphate (OMP) to uridine 5'-monophosphate (UMP), most probably through a carbanion intermediate and lasting about 50 milliseconds. During crystallographic studies of ligand binding, we discovered the slow (several days to completion) hydrolysis of 6-CN-UMP to 6-OH-UMP (BMP), the most potent inhibitor of ODCase known. Interestingly, the only obvious mechanism for this reaction seems to be a nucleophilic substitution.

Three independent approaches have confirmed this novel reaction: First, an electron density map (1.45 Å) from a crystal of ODCase incubated with 6-CN-UMP at room temperature for 2 months clearly identifies the compound at the active site as BMP. Second, MS analysis of ODCase freshly mixed with 6-CN-UMP shows a peak consistent with the presence of an ODCase-6-CN-UMP complex; after 7 days incubation, the spectrum displays a peak corresponding to an ODCase-BMP complex. Third, kinetic studies, taking advantage of the very low dissociation constant of BMP, confirmed the transformation. As 6-CN-UMP is converted to BMP, the product binds (almost) irreversibly to the enzyme's active site rendering it inactive. We conclude ODCase is catalyzing the conversion of 6-CN-UMP into BMP. Experiments to trap the 6-CN-complex of ODCase and to elucidate its crystal structure are in progress.

Keywords: enzyme catalysis, biomacromolecules, enzyme structure function

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Structural Basis for the Methylation Mechanism in Methyl-Transferase BchU Involved in Bacteriochlorophyll *c* Biosynthesis

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An S-adenosylmethionine (SAM)-dependent methyltrasferase, BchU, is an enzyme in bacteriochlorophyll *c* (Bchl *c*) biosynthetic pathway, and catalyzes methylation at C-20 position of chlorin moiety. To shed light on the methylation mechanism underlying the Bchl *c* biosynthesis, we have determined the crystal structures of BchU and its complex with SAM.

Recombinant BchU from *Chlorobium tepidum* was overproduced in *E. coli*, purified, and crystallized. We collected diffraction data using synchrotron radiation at SPring-8 and determined the crystal structure at 2.3 Å resolution (R-factor=0.24, R_{free}=0.28). In addition, we also determined the BchU structure in complex with SAM at 2.6 Å resolution (R-factor=0.21, R_{free}=0.26). The structure of BchU consists of two domains; N-terminal domain and C-terminal domain. The N-terminal domain is involved in dimerizaion and the C-terminal domain has a typical Class I motif. The SAM binds to Glu147, Asp175, Asn200, Asp227, Cys242 and Arg243. The location and orientation of the SAM help define the second substrate (a precursor of the Bchl *c*) binding site. These structural features and analysis of putative substrate-binding pocket provide invaluable information for the methylation mechanism of BchU.

Keywords: methylases, enzymatic reaction mechanisms, substrate binding