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The *E. coli* PDHc E1 Component Complex with a Reaction Intermediate Analogue

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The thiamin diphosphate (ThDP) dependent E1 component of the pyruvate dehydrogenase multienzyme complex (PDHc) catalyzes the decarboxylation of pyruvate and subsequent acetyl transfer to a lipoyl-lysine residue from the E2 component. Biochemical studies of the H407A E1 variant clearly indicated the importance of this residue to the overall reaction of the multienzyme complex. The specific activity of this variant is only 0.15% with respect to the native enzyme. In the native E1 crystal structure the loop region containing the residue H407 was unobserved due to disorder. Superposition of the E1 component and yeast transketolase (TK) structures indicates a general structural similarity and it is clear that if this region becomes ordered as in TK, the H407 residue can come very close to the ThDP and can interact with substrate or reaction intermediates. The crystal structures of the native and H407A variant of *E. coli* PDHc E1, both with a reaction intermediate analogue in its active site, have been determined to a resolution of 2.1 and 1.85Å respectively. Comparison of these two structures clearly indicates that the presence of the substrate analogue in the active site induces conformational changes in its vicinity.

Keywords: thiamin diphosphate, pyruvate dehydrogenase, E1 component

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Crystal Structure of Chitin Binding Domain of Chitinase A1

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The crystal structure of the chitin-binding domain (ChBD) of chitinase A1 (ChiA1), from *B. circulans* WL-12 has been determined. ChiA1 is a glycosidase that hydrolyzes chitin, and ChBD, ranging from Ala^{655} to Gln^{699} located at the C-terminal, binds specifically to insoluble chitin.

The diffraction data of the $ChBD_{ChiA1}$ crystal were collected at BL44XU at SPring-8, Japan, with the resolution of 0.95 Å. The phase was determined by the molecular replacement method using the structure previously determined by NMR as the model.

$ChBD_{ChiA1}$ has a compact and globular structure with the topology of a twisted β-sandwich. The overall topology is similar to that of the cellulose-binding domain (CBD) of *Erwinia chrysanthemi* endoglucanase Z (CBD_{EGZ}). However, $ChBD_{ChiA1}$ lacks the three aromatic residues (Trp^{18}, Trp^{43}, and Tyr^{44} in CBD_{EGZ}), aligned linearly and exposed to the solvent, which interact with cellulose. Mutation studies suggested that the loop region containing Trp^{687} interact with chitin. Moreover, $ChBD_{ChiA1}$ is detached from chitin by decreasing the pH value in solution from 4 to 3, probably because the charge in the side-chain of Glu^{688} is involved in the chitin-binding. Therefore, the binding mechanism of $ChBD_{ChiA1}$ is expected to be different from that proposed for CBDs.

Keywords: atomic resolution crystallography, glycosyl hydrolases, NMR

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Characterization of TenA from *Bacillus subtilis*: A Thiaminase II

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The biosynthesis of thiamin pyrophosphate has been the focus of considerable effort over the past decade; most of the proteins involved in assembly, salvage, transport and degradation have now been identified and in many cases structurally and mechanistically characterized [1]. A conspicuous exception is TenA, which in *B. subtilis* is part of the thiazole biosynthetic operon. TenA is known to be strongly repressed by thiamin [2], suggesting TenA may have a role in thiamin biosynthesis or metabolism. The structure of TenA alone and in complex with 4-amino-2-methyl-5-hydroxymethyl-pyrimidine have been determined to 2.6 Å and 2.5 Å, respectively. It has also been demonstrated that TenA has thiaminase II activity. The TenA structure suggests that the degradation of thiamin by TenA likely proceeds via the same addition-elimination mechanism described for thiaminase I [3]. While the chemical reaction catalyzed by thiaminases is well defined, the biological function is not yet clear. The over expression of TenA in *B. subtilis* results in an increase in the secretion of the degradative enzymes subtilisin, neutral protease and levansucrase [4], providing evidence of a possible relationship between TenA and the Deg proteins. DegS undergoes autophosphorylation in response to an unkown signal. It is tempting to speculate that this unknown signal may in some way be due to the binding and/or degradation of thiamin by TenA.

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Keywords: thiamin biosynthesis and degradation, enzyme mechanism, transcriptional activator

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Crystal Structure of Inhibitor-bound Mouse Cytidine Deaminase at 1.5 Å

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Cytidine deaminase (CDA) catalyses the deamination of cytidine and deoxycytidine to uridine and deoxyuridine. Two types of CDA, dimeric and tetrameric CDAs, have been classified [1,2]. The dimeric CDA has two cysteine and one histidine residues liganding a zinc ion at the active site, whereas the three residues are all cysteine in the tetrameric CDA. Arg56 of the tetrameric CDA from *Bacillus subtilis* partly neutralises the negative charge of the cysteine [2,3].

The inhibitor-bound structure of the tetrameric mouse CDA has, surprisingly, revealed the corresponding residue, Arg68, in two alternate conformations. While in the first conformation Arg68 forms hydrogen bonds with two of the zinc-binding cysteine residues, in the second conformation these hydrogen bonds are abolished. Although hydrogen bonds are important for maintaining zinc reactivity [3], the absence of it in the second conformation, conversely, can facilitate product dissociation by increasing negative charge donation from cysteine to the zinc ion, hence weakening the zinc–product interaction. Furthermore, the nearby Gln72 dyad, formed by Gln72 from two adjacent subunits, interacts with Arg68 in the second conformation, suggesting an allosteric cooperativity between the two subunits.

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Keywords: cytidine deaminase, alternate conformation, product dissociation

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***Arabidopsis thaliana* Acyl-CoA Oxidase 1 in Complex with AcetoAcetyl-CoA**

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