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Structure of Monomeric NADP-isocitrate Dehydrogenase: an Open Conformation

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Both monomeric and dimeric NADP-dependent isocitrate dehydrogenase (IDH) catalyze the oxidative decarboxylation from 2R,3S-isocitrate to yield 2-oxaloglutarate. Monomeric NADP-specific IDHs have been identified from about 50 different bacteria, whereas, dimeric NADP-dependent IDHs are diversified in both prokaryotes and eukaryotes. We have constructed the phylogenetic tree based on amino acid sequences of all bacterial monomeric NADP-IDHs. This is done to get an idea of evolutionary relationship. It is important to solve the structures of IDH from various species to correlate with its function and evolutionary significance. So far, only two crystal structures of substrate-bound (NADP or isocitrate) NADP-dependent monomeric IDH from *Azotobacter Vinelandii* (AvIDH) have been solved. Here, we are reporting for the first time the substrate free structure of monomeric IDH from *Corynebacterium glutamicum* (CgIDH) in the presence of Mg^{2+}. The 1.75 Ǻ structure of CgIDH-Mg^{2+} showed distinctly an open conformation in contrast to the closed conformation of Av-IDH-isocitrate/NADP complexes. The changes in fluorescence intensities of CgIDH in the presence of isocitrate or NADP indicate the conformational change. The conformational changes observed in fluorescence studies agree with the structural observation. Fluorescence results also suggest a low energy barrier between CgIDH with isocitrate or NADP resulting into easy access of the other substrate to perform the catalytic reaction. In CgIDH, the amino acids corresponding to the *E. coli* IDH phosphorylation-loop is alpha-helical compared to the more flexible random-coil loop in *E. coli*. This more structured region supports the idea that activation of CgIDH is not controlled by phosphorylation. This research is funded by the NSERC and SSI.

Keywords: crystal structure, monomeric IDH, fluorescence

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Study of Structural Change in SCOT upon Binding CoA

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Succinyl CoA:3-ketoacid transferase (SCOT, EC 2.8.3.5) allows cells to utilize ketone bodies [1] and individuals with low SCOT activity suffer from disease that can be fatal [2]. There has been decades of work done on this enzyme; it is known to form dimers and tetramers in solution [3] the substrates have been characterized [1] and the energetics of binding have also been calculated. Experiments in the 1990s showed that there is a large change in binding energy associated with a small region of the CoA molecule (pantoic acid domain) [4], and we are exploring what may contribute to this phenomenon.

SCOT is known to form a covalent thiolester intermediate with coenzyme A [5], and there is evidence for a structural change when CoA binds as noted by White et al. [6]. A structural change was postulated because SCOT is more readily inactivated by DTNB binding when the enzyme is bound to CoA. The specific cysteine being labeled was identified by Rochet [7] to be Cys28. We have studied the importance of this residue using site directed mutagenesis, kinetics as well as X-ray crystallography. The mutants constructed are C28S, C28A and C28W. C28A and C28S have be crystallized in P21 with dimensions a=63 Å, b=263 Å, c=59 Å, β=110º and both have diffracted to better then 2.3 Å.

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The Structure of *Anaerobiospirillum succiniciproducens* PEPCK Reveals an Important Loop

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The 2.2 Å resolution crystal structure of the enzyme phosphoenolpyruvate carboxykinase (PCK) from the bacterium *Anaerobiospirillum succiniciproducens* complexed with ATP, Mg^{2+}, Mn^{2+} and the transition state analogue oxalate has been solved. The 2.4 Å resolution native structure of *A. succiniciproducens* PCK has also been determined. It has been found that upon binding of substrate, PCK undergoes a conformational change. Two domains of the molecule fold towards each other, with the substrates and metal ions held in a cleft formed between the two domains. This domain movement is believed to accelerate the reaction PCK catalyzes by forcing bulk solvent molecules out of the active site. Although the crystal structure of *A. succiniciproducens* PCK with bound substrate and metal ions is related to the structures of PCK from *Escherichia coli* and *Trypanosoma cruzi,* it is the first crystal structure from this class of enzymes that clearly shows an important surface loop (residues 383 to 397) from the C-terminal domain, hydrogen bonding with the peptide backbone of the active site residue Arg60. The interaction between the surface loop and the active site backbone, which is a parallel β-sheet, seems to be a feature unique of *A. succiniciproducens* PCK. The association between the loop and the active site is the third type of interaction found in PCK that is thought to play a part in the domain closure. This loop also appears to help accelerate catalysis by functioning as a 'lid' that shields water molecules from the active site.

Keywords: phosphoenolpyruvate carboxykinase, anaerobio-spirillum succinici producens, domain movement

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Structural Analysis of *Listeria* Neutral Sphingomyelinase (SmcL)

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Pathogenic *Listeria* secrete phospholipases that help mediate disruption of phagocytic vacuoles, promoting bacterial intracellular replication. One of these enzymes, SmcL from *Listeria ivanovii*, is a member of the family of Mg^{2+}-dependent neutral sphingomyelinases (nSMases). These enzymes disrupt membranes by catalysing the hydrolysis of sphingomyelin (SM) to ceramide and phosphocholine. SmcL shares >50% identity with *S. aureus* nSMase (β-toxin) [1] and shows distant sequence homology to mammalian nSMases. In humans and other mammals, ceramide generation by SMases initiates a signalling pathway implicated in a number of important cellular responses including apoptosis, the stress-response, cell differentiation and cell proliferation. All the members of the nSMase family, whether bacterial or eukaryotic, appear to have a conserved active site, and likely a conserved overall fold [2].

Single crystals of *Listeria ivanovii* SmcL are available. These crystals diffract X-rays to >1.8 Å at synchrotron radiation sources and display trigonal symmetry (space group $P3_{1/2}21$). Detailed analysis of the SmcL structure will provide details of an enzymatic mechanism with relevance to bacterial pathogenesis and the understanding of mammalian cell signalling.

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