HIV INTEGRASE: FROM STRUCTURE TO DRUG DESIGN

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HIV-1 integrase catalyzes the insertion of viral DNA into the human chromosome, and as such, it is a target for the development of new anti-HIV drugs. Structural studies of HIV-1 integrase have been limited due to its insolubility. We have engineered a soluble, functional integrase by introducing five point mutations, and have solved the structure of the viral DNA binding core and C-terminal domains to 2.8 Å resolution. The Y-shaped, dimeric molecule reveals a putative DNA binding region consisting of residues contributed by both monomers of the dimer. This implies that a dimer is the minimal DNA binding unit. A kink at T210 occurs at a proteolytic cleavage site, suggesting a functional flexibility in the molecule that may be crucial for integration. The C-terminal domain is an SH3-like fold, and provides the majority of the crystal contacts, consistent with the role of the domain in oligomerization of integrase. Based on this structure, we are outlining new strategies to discover novel drug leads targeting integrase.

Keywords: HIV INTEGRASE DNA BINDING

ENZYMES: EVOLUTION OF FUNCTION FROM A STRUCTURAL PERSPECTIVE

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As more gene sequences are determined, the demand for better methods to predict protein function from sequence grows. Currently the only reliable approach is to predict function by recognising sequence and/or structural homology to a related protein, whose function is known. An overview will be presented of our current knowledge of how sequences, structures and functions evolve. In particular, we will consider the evolution of enzyme function, the evolution of enzyme function within homologous families(1), and the evolution of biochemical pathways(2,3). Approaches to capture the structures data relating to enzyme active sites as 3D templates will be described (4). An analysis of our results so far will be presented.

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Keywords: ENZYMES FUNCTION EVOLUTION

EVOLUTIONARY FOOTPRINTS FROM ANCIENT TIMES: THE NOVEL USE OF COMMON FOLDS IN THE BIOSYNTHETIC PATHWAY FOR COBALAMIN

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The evolution of a new enzymatic activity from an existing functional enzyme is universally accepted as a way that nature adapts to new opportunities. Although there are clear cases where this has occurred in recent years, such as the development of pathways to utilize chemical compounds that have never existed in the biosphere, there is also good reason to believe that this has been a common strategy since the beginning of life. In most cases the footprint of sequence similarity has long been lost through the accumulation of point mutations and genetic rearrangements so that the only vestige of similarity resides in the structural fold. The latter remains since every evolutionary step must proceed through a stable folded protein. Thus the structural study of ancient biosynthetic pathways can provide insight into the folds that were present at the time of their inception. Recent structural studies of the enzymes in the cobalamin biosynthetic pathway reveal that several common protein folds have been adapted to meet the novel enzymatic problems presented by the synthesis of nature's most complex cofactor. The structures of adenosylcobinamide kinase/adenosylcobinamide phosphate guanylyltransferase, ATP:corrinoid adenosyl transferase and L-threonine-O-3-phosphate decarboxylase will be discussed.

Keywords: ENZYME EVOLUTION COBALAMIN BIOSYNTHETIC PATHWAYS

MECHANISM OF SUCCINYL-CoA SYNTHETASE

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Succinyl-CoA synthetase (SCS) catalyzes the reversible reaction succinyl-CoA + NDP + P_i = succinate + CoA + NTP where N is adenosine or guanosine. SCS consists of two different subunits, α and β. During the reaction, a histidine residue of the α-subunit is transiently phosphorylated. The crystal structure of SCS showed that CoA binds to the α-subunit with the phosphorylated histidine residue approximately 7 Å from the thiol group of CoA. The structure of the complex of SCS with ADP-Mg^{2+} proved that the nucleotide binds to the predicted site in the 'ATP-grasp' fold of the β-subunit, however 35 Å away from where the active site histidine residue is seen in the structure. We postulated that the loop that includes the active site histidine residue flips to shuttle the phosphoryl group between site I, where CoA and, presumably, succinate bind, and site II where nucleotide binds. Our most interesting mutation to date is the change of a glutamate residue in site II to alanine, which was predicted to destabilize only the binding at site II. The mutant protein showed very low enzymatic activity, and, surprisingly, in our assay it could not be phosphorylated by succinyl-CoA and inorganic phosphate or by nucleotide triphosphate. The structure showed that two residues in site-I, Cys 123α-Pro 124α, were in a very different conformation than in the wild-type enzyme. We postulate, when in this conformation the protein cannot bind succinyl-CoA for phosphorylation by succinyl-CoA and inorganic phosphate.

Keywords: MECHANISM, ENZYME, PHOSPHORYLATED HISTIDINE