in the T-state (inactive; NADH bound). Notably, in the unregulated Type I CS these same residues form a stable folded region composed of two large helices that are a key part of the binding site for acetyl-CoA. Kinetic analyses of both types of CS indicate a similar catalytic mechanism, suggesting that in the active R-state of E. coli Type II CS, the conformation of residues 262-298 will be similar to that seen in the unregulated Type I enzymes. This uniquely evolved functionality switch in the Type II CS provides the opportunity to study a novel aspect of the protein folding problem, namely how to engineer the property of reversible instability into a formerly stably folded region. We have specifically designed and determined the structures of variant enzymes to gain a better understanding of the mechanistic features of this allosterically controlled regulatory folding/unfolding system. This has resulted in a series of stepwise structural snap shots of the refolding process in going from the T to R-states in Type II CS. Supported by the Natural Sciences and Engineering Research Council of Canada.

Keywords: enzyme allostery, protein folding, enzyme regulation

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Crystal structure analysis of human membrane integrated protein leukotriene C_4 synthase

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Human membrane integrated protein is one of the most exciting target for protein crystallographers, due to the difficulties in protein preparation as well as crystallographic work. In the year 2007, the structures of three human membrane proteins were determined crystallographically first. Leukotriene C_4 synthase (LTC_4S) is one of the three human membrane integrated proteins. LTC_4S, which is a membrane integrated protein existing in nuclear membrane, catalyzes the conjugation between leukotriene (LT) A_4 and glutathione (GSH) to form LTC_4. LTC_4 and its metabolites LTD_4 and LTE_4 are components of slow reacting substance of anaphylaxis, and they are called cysteinyl leukotrienes (Cys-LTs) because they have a cysteine moiety commonly. Cys-LTs are lipid mediators involved in smooth muscle constriction and inflammation, particularly in asthma. LTC_4S is the membrane protein responsible for the biosynthesis of Cys-LTs and a potential target for drug discovery. The crystal structure of human LTC_4S was determined at 3.3Å resolution using the recombinant LTC_4S. We established the over-expression system using *Schizosaccharomyces pombe*, and we also used the expression system for the preparation of the selenomethionine derivative of LTC_4S with a Leu121Met mutation for the MAD phase calculation as well as the native LTC_4S with a histidine tag. In the crystal structure LTC_4S forms trimer structure, and there is a V-shape substrate binding cleft between two adjacent monomers. GSH, which is one of the substrates, was co-crystallized with LTC_4S, bound at the upper space of the V-shape cleft. Based on the crystal structure, we proposed the acid-base catalytic mechanism, and Arg31 from a monomer and Arg104 from the other monomer exert the acid and base, respectively.

Keywords: membrane protein structures, X-ray crystallography of biological macromolecules, drug targets

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Structural insights into substrate specificity of isomaltase from *Saccharomyces cerevisiae*

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Oligo-1,6-glucosidase (dextrin 6-α-D-glucanohydrolase; EC 3.2.1.10) generally hydrolyzes nonreducing terminal α-1,6-glucosidic bonds of isomaltooligosaccharides and α-limit dextrin more rapidly than those of isomaltose. However, oligo-1,6-glucosidase from *Saccharomyces cerevisiae* (isomaltase) preferentially hydrolyzes isomaltose and weakly acts on isomaltooligosaccharides. To understand the difference in substrate specificity between isomaltase and other oligo-1,6-glucosidase from a structural view point, the crystal structure of isomaltase and isomaltase complexed with maltose as a competitive inhibitor were determined. Isomaltase was crystallized by the hanging drop vapor diffusion method using PEG3350 as a precipitant. An inhibitor complex was cocrystallized under the same conditions in the presence of 0.2 M maltose. Data sets for structural analysis were collected on synchrotron radiation to 1.6 Å resolution for the native and maltose complex. The crystals belong to the monoclinic space group *C*2 with the cell dimensions a=95.7 Å, b=115.4 Å, c=61.8 Å, β=91.2° and one 67 kDa molecule per asymmetric unit. The Overall structural features of isomaltase are similar to those of other GH family 13 enzymes such as α-amylases and *Bacillus cereus* oligo-1,6-glucosidase. It consists of three domains containing a $(\beta/\alpha)_8$ barrel structure. This reveals that the entrance of the active site cleft is narrowed by Tyr158, His280, and a loop between 310 to 315, thus disaccharide binds most efficiently in the active site for hydrolysis. The glucose residue of the nonreducing end is buried deeply at the bottom of the cleft, and tightly bound by nine hydrogen bonds and a stacking interaction with Tyr72. However, no structural change is observed before and after maltose binding.

Keywords: glycosyl hydrolases, isomaltase, substrate specificity

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Mechanism for formation of Arg-AMP in help of tRNA on the basis of structure of ArgRS, tRNA and ATP

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In arginyl- and glutaminyl-tRNA synthetases (ArgRS, GlnRS) from investigated all species, no ATP-PPi exchange reaction is observed in the absence of cognate tRNA and in the presence of their tRNA treated with periodate. The pyrophosphorolysis reaction of synthetic Arg-AMP is also not observed in the absence of tRNA. The detailed mechanism through which 2'OH of the ribose of the 3' end Ade76 of the cognate tRNA accelerates these reactions has been remained