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CRYSTALLIZATION STRATEGY FOR A SUCROSE TRANSPORTER AND STRUCTURAL STUDIES OF ENZYMES FROM THE STARCH AND SUCROSE METABOLISM

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Carbohydrates are important in the modern society. There is a growing need for carbohydrate compounds of all sorts from simple short oligosaccharides to large polymers. Even though some of these can be made by classic organic chemical synthesis, enzymes from the relevant metabolisms are obvious tools for the production of polymers and oligomers. Especially enzymes that use cheap and renewable substrates such as sucrose are of interest. The availability of recombinant enzymes also opens the possibility of obtaining rationally designed variants with tailored functions. Rational design of variants however requires the knowledge of the 3-dimensional structures of the enzyme in question. The aim of the project is detailed understanding of the reaction mechanisms of the following enzymes from the starch and sucrose metabolism: amylosucrase, glycogen synthetase, sucrose phosphorylase, invertase and dextran sucrase. We also aim at solving the structure of the E. coli sucrose transporter. Results presented will be on the crystallization attempts of the sucrose transporter, the cloning and expression of glycogen synthase and invertase, the progress in structure determination of sucrose phosphorylase and the structures of a number of complexes between amylosucrase and saccharides.

Keywords: SUCROSE MATABOLISM GLYCOSULTRANSFERASE STARCH

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STRUCTURAL INVESTIGATIONS TOWARDS A CATALYTIC MECHANISM OF *E. COLI* AMINOPEPTIDASE P

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Aminopeptidase P (AMPP) is a proline-specific peptidase that cleaves the N-terminal amino acid residue from a polypeptide chain where the penultimate residue is proline. AMPP plays important roles in a wide variety of biological processes. One such role in mammals is the regulation of the nonapeptide bradykinin, a potent vasodilator. Inhibition of AMPP can reduce blood pressure and assist reperfusion of ischemic myocardial tissue. We have previously published structures of *E. coli* AMPP crystallised at pH 4, at pH 8, and in the presence of the dipeptide Pro-Leu, a product-like inhibitor. *E. coli* AMPP is a tetramer, each monomer being comprised of two domains. The structure of the C-terminal domain resembles that of creatinase, prolidase and methionine aminopeptidase (AMPM). AMPP contains a dinuclear manganese site similar to the dinuclear cobalt site present in AMPM. Whilst previous studies provided a putative mechanism of action for AMPP, they left open a number of questions including the basis of N-terminal specificity and of specificity for proline in the second position. We have undertaken studies including the structural determination of complexes with inhibitors and of site-directed AMPP mutants to further elucidate the mechanism of action of AMPP. The structures of these complexes and mutant forms provide the basis for a more detailed model of the catalytic mechanism of AMPP.

Keywords: **AMINOPEPTIDASE METALLOENZYME MECHANISM**

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STRUCTURAL AND FUNCTIONAL DIVERSITY WITHIN THE 4-OT FAMILY OF ENZYMES

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The 4-OT tautomerase superfamily is represented by 4-oxalocrotonate tautomerase (4-OT), 5-(carboxymethyl)-2-hydroxymuconate isomerase (CHMI), and macrophage migration inhibitory factor (MIF). Members of this superfamily share a mechanistic feature - an amino-terminal proline as the general base in keto-enol tautomerization. The 4-OT monomer is distinguished by its small size (62 a.a.) and simple (β-α-β) fold. 4-OT and most of its homologues are hexameric while CHMI and MIF are trimeric. The diversity in the 4-OT superfamily suggests that nature used these short sequences as building blocks to create new structures and activities. In addition to Pro-1, Arg-11 and Arg-39 play key roles in binding the substrate at opposite ends of the active site.
BLAST searches have identified over 50 homologues of 4-OT. Multiple sequence alignment guided by key active site and structural residues allowed us to identify at least five subfamilies within the 4-OT family.
We have crystallized representative members from each subfamily, including the homologues from *Helicobacter pylori* (DmpI), *Bacillus subtilus* (YwhB), and *Escherichia coli* (YdcE). Native and inhibitor-bound YdcE crystal structures have been determined to 1.35 Å resolution. The protein's oligomeric state is a dimer, rather than the typical hexamer. Crystal structures of native and inhibitor-bound YdcE, as well as the homologues from *Helicobacter pylori* and *Bacillus subtilus* will be presented.

Keywords: **PROTEIN FOLDING, TAUTOMERASE, SUPERFAMILY**

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DOMAIN MOVEMENTS INDUCED BY SUBSTRATE BINDING IN OMP DECARBOXYLASE

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Orotidine 5'-monophosphate decarboxylase (ODCase) catalyses the decarboxylation of orotidine 5'-monophosphate to uridine 5'-monophosphate. We have earlier determined the structure of ODCase from *E. coli* complexed with the inhibitor 1-(5'-phospho-beta-D-ribofuranosyl)barbituric acid (BMP); here we present the 2.5 A structure of the uncomplexed apo enzyme, determined from a crystal that was a perfect merohedral twin. The structure determination and refinement will be described. Å structural analysis and comparison of the two structures of the *E. coli* enzyme show that binding of the inhibitor is accompanied by significant domain movements of approximately 12 deg. around a hinge that crosses the active site. Hence, the ODCase dimer, which contains two active sites, may be divided in three domains: a central domain that is fixed, and two lids which independently move 12 deg. upon binding. Corresponding analyses of the two *S. cerevisiae* ODCase structures (with and without BMP) and the *Methanobacterium thermoautotrophicum* ODCase structures (with and without 6-aza UMP) show very similar, but somewhat smaller domain movements. The domain movements seem to be initiated by the phosphoryl binding to the enzyme and can explain why the binding of the phosphoryl group is essential for the catalytic function.

Keywords: **OMP DECARBOXYLASE, TWINNING, DOMAIN MOVEMENTS**