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Keywords: endonuclease, catalytic mechanism, fold

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Non-catalytic domain of invertases: the key for oligomerization and specificity.

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Fructans are polymeric sugars derived from sucrose. They are most interesting because of their physiological characteristics, such as preventing colon cancer and dental caries, selectively stimulating the growth of bifidobacteria and lactobacilli, decreasing total cholesterol and triacylglycerol lipids in blood serum and promoting the absorption of calcium and magnesium ions. Therefore, the enzymes involved in fructans processing attract great biotechnological attention for the production of functional foods and pharmaceuticals. In particular, a detailed knowledge of the molecular mechanisms involved in substrate recognition, transfructosylating efficiency and product specificity of the enzymes used as catalyst for these processes is essential.

We have solved the crystal structure of three invertases from yeast. First, the *Schwanniomyces occidentalis* Invertase, complexed with long substrates, revealed for the first time that the ancillary domain plays a direct role in oligomerization and substrate binding [1], which is a unique feature that shed light on the molecular mechanism regulating specificity within the GH32 enzymes from eukariota. We report also the *Phaffia rhodozyma* Invertase structure [2], an atypical highly glycosylated enzyme, with an unique insertion in the sequence of the ß-sandwich that folds over the catalytic domain and is involved in a new oligomerization pattern conferring high stability to the enzyme. Finnally, we have studied the *Saccharomyces cerevisiae* Invertase, an enzyme reported to adopt different aggregation states upon changes in the environment. The crystal structure revealed a sophisticated mechanism of molecular interaction between subunits that form higher aggregates throughout further involvement of the ancillary domains. Our results assign a direct catalytic role to the supplementary ß-sandwich domain of these enzimes, the first time that such a role has been observed within GH32 enzimes.

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Structural and functional studies of *Staphylococcus aureus* Pyruvate Carboxylase

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Pyruvate carboxylase (PC) catalyzes the ATP-dependent transformation of pyruvate to oxaloacetate, which marks the first step in gluconeogenesis. Oxaloacetate is also an important intermediate in the tricarboxylic acid (TCA) cycle. Therefore, PC is considered an essential enzyme in intermediary metabolism. The structural architecture of PC consists of four domains, the biotin carboxylase (BC) domain, the carboxyltransferase (CT) domain, the biotin carboxyl carrier protein (BCCP) domain, and a novel PC tetramerization (PT) domain. It belongs to a group of biotin dependent enzymes where the biotin is covalently bound to BCCP, which swings between the distinct active sites on the BC and CT domains to carry out the catalysis. The recently discovered PT domain is essential for keeping the tetramer intact and mutations in this domain disrupt the tetramer in both human and *Staphylococcus aureus* PC. In terms of regulation, it is known that PCs from vertebrate sources are highly activated by acetyl-CoA, while PCs from prokaryotes have varying degrees of dependency on acetyl-CoA.

Structural and biochemical studies performed on the BC domain of this important enzyme will be presented. Through mutagenesis studies of four key residues that reside in the BC dimer interface of *Staphylococcus aureus* PC, we found that the BC dimer is very stable. Most of the mutations eliminated enzymatic activity, as well as disrupted the normal tetrameric state of PC in solution. This indicates that PC has a strong tendency to self-associate in its active, tetrameric form, especially at high concentrations of protein.

Keywords: enzyme, biotin-dependent carboxylase, metabolism

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Crystal structure of the catalytic domain of multidomain PHB depolymerase

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Polyhydroxyalkanoate (PHA) is a naturally occurring biodegradable biopolyester which is synthesized and degraded by microbes. PHA represents various physical properties depending on the chemical structure of monomer units, and it draws attention as an environment-friendly material. PHA is synthesized and stored as an energy-storage material in bacterial cells, and stabilized in an amorphous state, coated with fatty acids and proteins. This native PHA changes its structure to a partially crystallized state (denatured PHA) by removal of associated fatty acids and proteins. Specific PHA depolymerases acting on each types of PHA are known. Extracellular depolymerases are able to act on denatured PHA but not on native PHA, whereas intracellular depolymerases have the opposite specificity.

Most of known extracellular depolymerases act only on denatured PHA with short-chain-length monomer units (mainly *R*-3-hydroxybutyrate), so they are often called polyhydroxybutyrate (PHB) depolymerase. They are typically composed of three domains: catalytic, linker and PHB-binding domains. We have succeeded in determination of the crystal structure of the catalytic domain of an extracellular PHB depolymerase from *Ralstonia pickettii* T1 at 2.4 Å resolution by Au-SAD method, and refined its coordinates to 2.1 Å resolution. Crystals of the enzyme belonged to monoclinic space group $P2_1$, with cell dimensions a = 67.3 Å, b = 150.7 Å, c = 78.3 Å, β = 93.1˚, and contained four polypeptides in the asymmetric unit. Crystals of a gold derivative were obtained by quick-soaking using a solution containing $KAuCl_4$. Diffraction data were collected with X-ray of the