GLYOXYLATE REDUCTASE AS VERSATILE ENZYMATIC SYSTEM FOR PHARMACEUTICAL AND MEDICAL USE

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The chiral homogeneity of a chemical compound is the main prerequisite in safety and efficiency of drug substances and generation of single enantiomers of drug intermediates in pharmaceutical industry. Over the past several years there have been an increase wide variety of enzymes and bioengineered microorganisms used for biotransformation of chemicals with chemo- regio- and enantioselectivity [1]. The direct evolution – a combination of biochemistry, molecular biology, structural biology and bioinformatics predictions [2] can modulate enzyme stability, reactivity or substrate specificity. One example of enzymes used in pharmaceutical industry are enzymes from the D-2-hydroxyacid dehydrogenase (2HADH) family. They catalyze reversible reduction of 2-oxoacids to 2-hydroxyacids in an NAD(P)H dependent manner, playing a key role in metabolism of many organisms. One of the enzyme group from 2HADH family is glyoxylate reductase (GR) [3]. The representative of this group was first isolated from spinach leaves as early as in the 50's, showing the reduction of glyoxalic acid to glycolic acid using reduced pyridine nucleotides. The advantages of these enzymes have been recently recognized by pharmaceutical and biotechnological industry, considering a possibility of highly stereospecific biotransformation of α-ketoacids into homochiral α-hydroxyacids, as important industrial intermediates [4]. The glycolic acid, reduced by the enzyme into ethylene glycol, the smallest member of the α-hydroxy acid family, is nowadays obtained in a large-scale industrial process. Very recently, glycolic acid production through the glyoxylate cycle has been reported in bacteria and also in yeast (*S. cerevisiae*). A number of novel aplications of glyoxylate reductase have been lately discovered. These include the use of glyoxalate reductase/hydroxypyruvate reductase (GRHPR) enzymatic system as an independent prognostic marker in hepatocellular carcinoma (HCC) [5]. The GRHPR also interacts with the sodium-dependent vitamin C transporter-1 to regulate cellular vitamin C homeostasis [6].

Glyoxylate reductase plays an important role in human metabolism where it is responsible for removal of glyoxylate from liver cells. This enzyme is involved in genetic diseases like primary hyperoxaluria type II (PH2) and nephropathy diseases, where its deficiency is manifested in increased urinary oxalate levels, formation of kidney stones and renal failure. In the bacterial kingdom glyoxylate reductase prevents from accumulation of toxic methylglyoxal and influences many biochemical pathways.

The monomeric structure of the enzyme comprises two domains typical for NAD(P)-dependent dehydrogenases: the substrate-binding domain (SBD) and the nucleotide-binding domain (NBD). Several crystal X-ray structures of glyoxylate reductase from different species have been already solved, but only few of them were determined with the bound substrate and/or cofactor. Such structures are crucial in understanding the reaction mechanism and for predicting or designing the structures of new substrates for the enzyme.

The *Sinorhizobium meliloti,* a gram-negative bacteria, are in symbiosis with leguminous plant playing important role in converting atmospheric nitrogen into organic one (nitrogen fixation model). *S. meliloti* induce and infect plant's nodules providing its host with biologically assimilable forms of nitrogen and receiving, as a main carbon source (dicarboxylic acids e.g. malate, succinate, fumarate, and other primary metabolites).

We will communicate the structural study of two homodimeric glyoxylate reductases from *S. meliloti* (*Sm*GR1 and *Sm*GR2). We have solved the crystal structures for *Sm*GR1 and *Sm*GR2 with bound oxalate and NADPH and compared them with the structures of other glyoxylate reductases subfamily members: from *Rhizobium etli* (with L(+)-tartaric acid and NADP) and human (with (2*R*)-2,3-dihydroxypropanoic acid and NADPH). During the ligand and cofactor binding the catalytic domain rotates towards coenzyme-binding site, changing its structure from open to close conformation.

Using biochemical tools and kinetics approaches we have also shown that *Sm*GR1 and *Sm*GR2 possess substrate specificity to hydroxypuryvate, hydroxylphenylpyruvate and gloxylate, providing new insights into the potential pharmaceutical and medical uses of this family of enzymes.

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