

MS9-P11 Structure-Function-Analysis of proteins involved in the metabolic pathway of vitamin K acting as major pathogenic factors in *Staphylococcus aureus* infection

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In recent years the level of infections caused by human bacterial pathogens is rising. In Europe, attention has been drawn on infections acquired during hospitalization, specially caused by the methicillin-resistant *Staphylococcus aureus* (MRSA). Therefore, novel chemotherapeutic agents are urgently required, which solely target the bacteria and not the human host. Genome analyses indicated the presence of a functional vitamin K biosynthetic pathway consisting of the gene products MenA-F. Vitamin biosynthetic pathways are not present in humans and therefore inference with the host organism is not expected. In order to discover selective pro-drugs high resolution structural information about the respective enzymes participating in the biosynthetic pathway is required. In terms of structure based pro-drug design we target the proteins MenF, MenH and DHNA of the vitamin K metabolism in *S. aureus*, in order to obtain high-resolution structure information. Thus, genes of these proteins were cloned into pAKS-IBA-3 vector and expressed in *Escherichia coli*. Proteins were purified on affinity column containing the Strep-Tactin resin and impurities were removed using size exclusion and ion exchange columns. To investigate stability, homogeneity and secondary structure prior to crystallization experiments, dynamic light scattering (DLS) and circular dichroism (CD) were performed. Once verified the stability and homogeneity, initial crystallization trials were performed with DHNA using sitting drop vapor-diffusion mixing 1 μ L of protein (10 mg/ml) with 1 μ L of reservoir solution (100 mM HEPES-Na pH 7.0, 1 M lithium sulphate) in MRC Maxi 48-well plates at 20 °C. Protein crystals were observed after 2-3 days, reaching maximum dimensions within a week. The crystals were transferred to a cryo-protective solution (reservoir solution added 15% glycerol), flash-cooled in liquid nitrogen and initial diffraction data were collected applying a conventional rotating anode X-ray source. Preliminary CD and structural results show that 4-hydroxybenzoyl-CoA thioesterase (DHNA) consists of four repeating units (homo-tetramer) with approx. 22% of alpha helices, 49 % beta sheets, 4 % loops and 24.5 % random coil. The protein crystal diffracted to 2.6 Å resolution, belonging to the space group P2₁. Initial crystallization experiments for MenF and MenH are ongoing as well as the incorporation of selenomethionine for DHNA to solve the Phase problem, Up to date details will be presented.

Keywords: methicillin-resistant *Staphylococcus aureus* (MRSA), Vitamin K biosynthesis, Pro-drugs, Drug design, Crystallography

MS9-P12 Structural studies of β -lactoglobulin from various species

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β -Lactoglobulin (LG) is a small globular protein of m.w. 18-20 kDa, belonging to the lipocalin family. Lipocalins share fold in which a core element is 8-stranded β -barrel. Four flexible loops surround entrance to the binding pocket located in the interior of beta-barrel. Reversible opening and closing of EF loop attributed to pH changes regulates access to the binding pocket. At physiological conditions LG forms dimers with subunits related by 2-fold symmetry.

LG has been found in the whey fraction of milk of more than 30 species, but not in human milk. The physiological function of LG is not well recognized, probably it is a molecular transporter of hydrophobic compounds. Recently, increased attention is focused on lipocalins due to their potential utilization in clinical applications as molecular transporters. Such an application of lactoglobulins requires numerous modifications influencing their binding properties. Therefore, to gain better understanding of individual residues role in ligand binding, we have undertaken systematic structural and thermodynamic studies of LG from various species being natural modifications to this protein.

Most thoroughly studied LG is a bovine protein (BLG) isolated from cow milk, for which crystal structures of several complexes with various ligands are available. The most abundant BLG isoforms are A and B that differ at two positions: D64G and V118A. The milk of sheep contains three isoforms of LG (A, B and C) that compared to isoform B of BLG have six substitutions. Only one isoform of LG has been found to date in goat milk, it also differs from BLG-B at six positions.

In this work we present crystal structures of bovine, sheep and goat LG. It was found that in BLG substitutions of polar residues in positions 64 and 118 affected thermodynamic of ligand binding but not influence overall structure of complexes. Systematic distortion of the β -barrel was observed in sheep protein in comparison to BLG. Also, differences in distribution of electrostatic potentials on the molecular surface in the dimer interface and entrance to the binding pocket region have been observed. In crystal structures of goat LG unusual conformation of flexible EF loop has been found, indicating that opening and closing access to the binding pockets in LG dimer might be sequential and cooperative.

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Keywords: lactoglobulin, lipocalin