Crystallographic studies on Kdo transfer in the hyperthermophilic bacterium *Aquifex aeolicus*

H. Schmidt¹, J. R. Mesters¹, R. Hilgenfeld¹ and U. Mamat²

¹ Institute of Biochemistry, Center for Structural and Cell Biology in Medicine (CSCM), University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany

² Division of Structural Biochemistry, Research Center Borstel, Leibniz-Center for Medicine and Biosciences, Parkallee 4a/4c, D-23845 Borstel, Germany

Lipopolysaccharides (LPS) are characteristic components of the outer leaflet of the outer membrane (OM) of virtually all Gram-negative bacteria. LPS of wild-type bacteria display a common structural architecture consisting of three regions: the OM-embedded lipid A, the core region, and the O-specific polysaccharide [1]. The 8-carbon sugar 3-deoxy-D-manno-oct-2-ulosonic acid (Kdo) serves as the direct link between lipid A and the polysaccharide chain. It is the only conserved structural element found in almost all core regions investigated so far [2]. The inner-membrane-associated Kdo-transferase WaaA utilizes an activated CMP-Kdo as a substrate for the attachment of Kdo to the lipid A moiety. Since the Kdo-lipid A region of the LPS molecule is essential for cell survival and growth [1], all enzymes involved in Kdo-biosynthesis, -activation or -transfer represent potential targets for the design of novel antibiotic drugs.

WaaA from *Aquifex aeolicus* was successfully cloned, expressed, purified and crystallized. The crystals belong to the monoclinic crystal system with spacegroup P2₁ and unit cell parameters \( a = 132.28 \text{ Å} \), \( b = 45.92 \text{ Å} \), \( c = 144.03 \text{ Å} \) and \( \beta = 97.08^\circ \). They diffracted X-rays to a maximum Bragg spacing of 2.6 Å at the X13 beamline at DESY (Figure 1). We decided to apply the MIR technique for solving the phase problem. Hence, WaaA was crystallized in the presence of various heavy atom compounds. Furthermore, native crystals were pressurized with xenon gas. Three data sets of potential heavy atom derivatives and one data set of a potential xenon derivative were collected at the X13 beamline. Difference-Patterson analysis revealed no peaks for the heavy atoms or xenon. At the moment we perform more soaking experiments in order to successfully derivatize our crystals.

![Figure 1: A) WaaA crystal B) Diffraction image of a WaaA crystal.](image)

References


This project is being supported by the Deutsche Forschungsgemeinschaft (Me2741/1, Ma1408/2).