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Structure determination of the B-repeat, a domain of the invasion protein InlB. Maria Ebbes and Hartmut Niemann *Department of chemistry, Bielefeld University, Germany.*
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Internalin B (InlB) is a surface protein of the pathogenic bacterium *Listeria monocytogenes*. This protein binds the extracellular domain of the human receptor tyrosine kinase c-Met to induce uptake of the bacteria by phagocytosis into non-phagocytic cells. InlB consists of different domains, only one of which is involved in the binding of c-Met. We are working on the B-repeat, a domain in the middle of InlB, to which no function is allotted at the moment. We obtained a native data set with a resolution of 1.3Å. No obvious homologs exist, so we had to solve the phase problem experimentally. To this end, we used SeMet-crystals. From these crystals we measured a four-wavelength MAD data set at DESY, X12. The structure was solved with ShelxD. The crystals contain four molecules per asymmetric with one internal and the N-terminal Se-Met per molecule. ShelxD located five out of these eight Se atoms. Solvent flattening in ShelxE including the native data produced an excellent experimental electron density. Here we present the structure of the InlB B-repeat and its initial analysis including fold comparison and the prediction of binding sites for potential interaction partners.

Keywords: MAD phasing, protein structure, bacterial pathogenesis

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Phasing glycosyltransferase PimB' – *P*1 symmetry and radiation damage. Klaus Fütterer, Sarah Batt, Gurdyal S. Besra. *School of Biosciences, University of Birmingham, UK.*
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Phosphatidylinositol mannosides (PIMs) are glycolipids in the cell wall of *Mycobacterium tuberculosis* (and related species) that modulate the host immune response and help establish a long-lasting latent infection, a hallmark of the pathophysiology of tuberculosis. Decoration of the inositol ring of phosphatidylinositol (PI) with α-D-mannose occurs through consecutive action of a series of related mannosyltransferases of the GT-B superfamily of glycosyltransferases. The enzyme PimB' catalyses transfer of α-D-mannose from GDP-mannose to the 6-hydroxyl of the inositol moiety. Using recombinant protein of *Corynebacterium glutamicum*, a non-pathogenic relative of *M. tuberculosis*, we obtained plate-like crystals of PimB' that were in space group *P*1, diffracting up to 2.2 Å resolution with two copies of PimB' in the asymmetric unit. Phasing by molecular replacement, using low-identity search models of related glycosyltransferases, failed to produce an interpretable map, while the 10 μm-thin crystals did not tolerate heavy metal soaking. Crystals of SeMet-derivatised PimB' rapidly suffered from radiation damage, even in a much attenuated beam, limiting data redundancy and anomalous dispersion, while data completeness was restricted by unusable reflection profiles in two 60º segments over a 360º degree sweep. We eventually succeeded in phasing this structure through combining 4 individual SeMet-SAD data sets with data of PimB' bound to brominated GDP-mannose in a phasing run with SHARP. Map interpretation rested on phase improvement by NCS-averaging and matching experimentally found Se-positions, using SHELXD, with the Met-residues in a homology model of the conserved, Rossmann fold-like C-terminal domain of PimB'. Iterative model building and refinement could then proceed in a straightforward fashion.

Keywords: *Mycobacterium tuberculosis*, GT-B glycosyltransferase, SAD phasing

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Protein tags as phasing tools? Christian Große, Georg Michael Sheldrick. *Dept. of Structural Chemistry, Georg-August-Universität Göttingen, Germany.*
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During the last decade, several affinity protein tags have been developed, which are included as an additive protein sequence. Their highly specific strong affinity affords an one-step purification with minimal effect on biological activity. On the other hand, protein tags are often flexible and disordered in the crystal structure. However, tags are often cleaved and can not be used for experimental Phasing of macromolecules by anomalous dispersion. Those kind of experiments requires well ordered atoms in the crystal lattice. A preorganized protein tag with metal chelating properties can bind anomalous scatterers in a determinate way. As a welcome side-effect such protein tags could adopt stable conformation in crystals and help the crystallization process. Here we report polypeptides synthesized by solid phase peptide synthesis which may prove useful for both metal affinity chromatography and macromolecular phasing as well. A distinct secondary structure in terms of β-hairpins could be confirmed by circular dichroism spectroscopy and NMR. As expected folding becomes stronger and the melting point increases due to additional metal ions. Their chelating properties could be proved by Ni, Zn, Co and Cu affinity columns and some candidates show as high chelating power as a classical His tag peptide. Fusion proteins made of Maltose-binding protein (MBP) fused with our polypeptides were used to check IMAC behavior under real conditions and to validate crystal growth promotion. We observe improved crystallization in the present of metal ions and solved first crystal structures from native protein.

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Keywords: His-tag, experimental phasing, β-hairpin

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