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Investigation of the Mosaicity of a $Li_2SO_4 \cdot H_2O$ Crystal

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No single crystal is perfect; a real crystal can be approximated by mosaic blocs of small perfect crystals. This approach is the basis for extinction analysis in X-ray structure analysis.

Extinction is the weakening of the diffracted X-ray intensity due to multiple scattering in the crystal. Perfect crystals scatter according to the dynamical theory ($I \sim |F|$), but no real crystal is perfect. Ideal imperfect crystals, on the other hand, scatter according to the kinematical theory ($I \sim |F|^2$).

In most cases, the measured intensities of real crystals are in between both cases and an extinction correction is needed to fulfil the kinematic approach.

Present theories dealing with extinction corrections are based on the approach of a mosaic crystal and describe x-ray scattering in terms of kinematic approach using certain "correction terms" to implement the structure of a real crystal **[1, 2, 3]**. Most experimentalists use the extinction as black box. However, for a real structure refinement the parameters used for a certain extinction correction are typically not verified by the experiment.

The mosaic blocs within a real crystal are misorientated to each other and are affected by lattice strain. In addition both 3D shape and size of the blocs are not known. All these parameters can be determined by high-resolution x-ray diffraction techniques as know from semiconductor research performing ω- and ω-2θ-scans through certain reciprocal lattice points.

Our approach is to verify the validity of existing extinction theories by independent diffraction experiments with high resolution. In our present experiments we used a $Li_2SO_4 \cdot H_2O$ crystal plate. The measurements were performed at beamline D3 at Doris, Hasylab at DESY. Several reflections in Laue and Bragg geometry were measured.

The ω-scan is a rotation of the crystal by $\Delta\theta$, while the detector is fixed at 2θ and the ω-2θ-scan is a rotation of the crystal by $\Delta\theta$ and simultaneously a movement of the detector by $2\Delta\theta$. The determined parameters from the FWHMs of these scans can be used to determine extinction.

Finally the evaluated parameters are used for structural refinement and are compared with solutions achieved without the knowledge of the real structure.

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The structure of SapA lipoprotein discs

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Sphingolipid activator protein SapA is an essential non-enzymatic cofactor required for the breakdown of galactosylceramide by -galactosylceramidase within the lysosome. A defective SapA fails to activate the degradation of galactosylceramide, which in turn leads to globoid-cell leukodystrophy (Krabbe disease). The 1.9 Å crystal structure of the SapA in complex with 40 internally-bound detergent (LDAO) molecules provides a high-resolution view of a discoidal lipoprotein particle. In this complex the acyl chains of the detergent molecules form a highly ordered bilayer-like hydrophobic core surrounded by two copies of a protein belt. Saposin A lipoprotein discs exhibit limited selectivity with respect to the incorporated lipid, and can solubilize phospholipids, sphingolipids and cholesterol into discrete, monodisperse particles with mass of approximately 27 kDa. These discs may be the smallest possible lipoprotein structures that are stabilized by lipid self-assembly. The saposin A-lipid disc is most likely the effective substrate-presenting particle in galactosylceramide hydrolysis. We propose that saposin A activates the galactosylceramide hydrolysis reaction by solubilizing the target lipid in 3.2 nm particles, thus increasing the accessibility of the lipid headgroup to the enzyme active site.

Key words: lipoproteins, saposin, X-ray crystallography

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First prokaryotic LOX structure: interactions with phospholipids and membranes

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The biological role of lipoxygenases, non-heme iron dioxygenases responsible for a myriad of essential functions in eukaryotes, remains unexplained in prokaryotes, which generally lack polyunsaturated fatty acids. The first crystal structure of a prokaryotic lipoxygenase (Pa_LOX) from *Pseudomonas aeruginosa*, shows major differences with respect to eukaryotic enzymes such as the lack of the PLAT domain and the insertion, in the catalytic domain, of a pair of long antiparallel α-helices. An enlarged binding pocket showed a complete phosphatidylethanolamine molecule (with chains of 18 and 14 carbons in length). Five carbons, of the longest chain, approach the catalytic iron suggesting how the pentadienyl moiety is stabilized within the substrate binding pocket. Several crystal forms from the native Pa_LOX and two inactive mutants confirm the high affinity of the N-terminus towards the phospholipid polar moiety. In turn, the variability of the disposition of the two helices insertion suggests the exposure of the hydrophobic upper part of the substrate binding pocket towards the membrane during the capture of phospholipids. Measurements, performed using phospholipid containing vesicles by means of ITC and fluorescence spectroscopy, show a clear interaction of Pa_LOX with certain model membranes.

Eukaryotic lipoxygenases perform a crucial role in the pathways involved in inflammatory processes against pathogen infection. In addition, for these enzymes it has also been reported a high degradation activity against the membranes of some cellular organelles. All this, and taking into account the capability of Pa_LOX to interact directly with membrane phospholipids, without any further facilitator, invites to hypothesize that Pa_LOX was preserved as a secreted enzyme to interfere with the host immune response and/or to assist in the degradation of membrane barriers.