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Structural Studies on MsmE, a Sugar Binding Lipoprotein from *S. pneumoniae*

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Streptococcal organisms share a well-conserved, binding protein dependent, Multiple Sugar Metabolism (msm) system which is responsible for the transportation and metabolism of multiple sugars. This system is comprised of 8 proteins including 2 membrane proteins (MsmF and MsmG), an ATP-binding protein (MsmK) and the 45KDa sugar-binding lipoprotein MsmE [1].

Both native and selenomethionine derivatised MsmE were crystallised in space group $P6_122$. Selenomethionine derivative crystals showed diffraction to 2.5Å and 3-wavelength MAD data were collected at SRS, Daresbury. Selenium sites (19/22) were found using SnB [2] with peak data and site refinement and phasing using Sharp/AutoSharp [3] using MAD data along with a 3Å native dataset.

The initial model shows a two domain periplasmic binding protein-like fold and model-building and refinement is continuing.

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Keywords: MsmE, crystallography, selenomethionine derivatives

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Macromolecular Crystallography at Room Temperature: Wavelength Dependence Radiation Sensitivity and Damage

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Biological samples are known to be strongly radiation sensitive and suffer from radiation damage during room temperature X-ray data collection even at cryogenic temperatures (100K). One of the factors affecting the rate of radiation damage on biological samples is mainly to use of the wavelength for 3D structural analysis. Here we attempt to determine the wavelength dependence effects of radiation on biological samples. Our results show that radiation sensitivity presents a direct dependence with the wavelength. Several derivatives from Hen Egg White Lysozyme crystals were grown in the gel by the Counter Diffusion Method in standard Hampton Research Granada boxes. Synchrotron radiation data were recorded at the X6A beam line of the National Synchrotron Light Source at room temperature. A threshold wavelength was determined for each sample derivative for which radiation sensitivity and damage significantly increased.

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Keywords: macromolecular crystallography, radiation damage, wavelength

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Solution Scattering Studies of Xylanase XYNII from *Trichoderma longibrachiatum*

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Xylanase (endo-1,4-β-xylan xylanohydrolase, EC 3.2.1.8) is an enzyme catalyzing the hydrolysis of β-1,4-D-xylosidic linkages of xylan. *Trichoderma longibrachiatum* has two different xylanases: XYNI and XYNII. Xylanase XYNII is a small protein (21 kDa; 190 a.a).

X-ray solution scattering measurements of XYNII were performed on the EMBL X33 beam line at DESY, Hamburg (Germany). The SAXS/WAXS camera was used to cover the scattering vectors $0.16 < s < 9.2$ nm^{-1}, with $s = 4\pi\sin\theta/\lambda$, where the 2θ is the scattering angle and $\lambda = 0.15$ nm. The radius of gyration, forward scattering and distance distribution functions were calculated using the program GNOM [1]. The low resolution structure was restored from experimental data by programs: DAMMIN [2] and GASBOR [3].

The radii of gyration R_G measured by SAXS were 1.65 nm (pH 3.0) to 1.72 nm (pH 10). The experimental scattering curve was compared to this evaluated from crystal structure. The low resolution structure and also the domain structure (chain compatible spatial distribution model) will be presented.

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Keywords: small-angle X-ray scattering, wide-angle scattering, xylanase

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Towards the Structure Determination of the HtrA1 Protein from *Staphylococcus aureus*

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The HtrA1 protein of *Staphylococcus aureus* has a high degree of sequence homology to members of the HtrA/DegP family from Gram-negative bacteria. Expression of this protein is induced in response to heat shock or secretion stress signals in *Bacillus subtilis* [1]. HtrA/DegP shows a temperature dependent "switch" from chaperone to (serine)protease activity [2], a function that has also been proposed for the corresponding protein in Gram-positive bacteria [3]. We are expressing HtrA1 with a view to crystallization and structure determination. A His-tagged wildtype HtrA1 protein was expressed, containing an enterokinase cleavage site in a flexible linker region. Initial purification revealed that the protein probably undergoes self-cleavage during removal of the His-tag. For this reason the serine residue of the proteolytic site was mutated to alanine. This mutant protein was purified and appears stable during the process of enterokinase clevage and subsequent purification. Using size exclusion chromatography the HtrA1(SA) protein shows an elution profile corresponding to a monomeric molecule. Crystallization trials are currently under way, and promising conditions have been determined.

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Keywords: DegP, protease, chaperone

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Advanced High-throughput Platforms for Protein Crystallography

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In recent years, the area of protein crystallization has been subject to fundamental developments. The demand for sophisticated and diversified platforms, especially with regard to optical properties, multiple screening capabilities, and suitability for small sample volumes, has resulted in the creation of highly specialized, multi-faceted products to meet the diverse requirements of automated high-throughput approaches. Combined with additional necessities for more

efficient drop inspection and analysis, these technological advancements have initiated the development of microplates with low birefringent background (LBR plates) to allow more effective use of polarized light in protein crystal detection. LBR plates are especially beneficial for identification of crystals out of focus, very small microcrystals, microcrystals hidden in or resembling precipitate or phase seperation and crystals located at the edge of droplets or crystallization wells.

As an alternative to classical microplates, plastic microstructured devices for liquid-liquid diffusion crystallography offer the benefits of low protein and reagent consumption, ease of handling and time conservation. Further advantages of plastic microstructures devices are a broad selection of available raw materials and surface treatments as well as reasonable costs of manufacture.

Keywords: high throughput crystallography, polarized light microscopy, microfluidics

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Lactate Dehydrogenases from Extremophile Organisms: Clues for Radio-resistance?

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Lactate dehydrogenases (LDH) catalyse the last step of glycolysis in which pyruvate is reduced by NADH to lactate. Our structural study of LDH from *D. radiodurans* finds its place in a broader project aimed at investigating the effect of ionising radiation on many enzymes of the malate/lactate dehydrogenase family from extremophile organisms (thermophiles, psychrophiles, halophiles and radio-resistants).

Ionising radiation, including synchrotron radiation, has specific effects on structure, activity and stability of biological macromolecules and represents at the same time the cause of the damage and the tool to study it [1].

In order to better understand how molecular adaptation to extreme environments is achieved, and if it confers radio-resistance, the structure of LDH from *D. radiodurans* has been solved, both in its native form and in complex with the allosteric cofactor fructose 1,6-biphosphate (FBP). A structural comparison with LDHs from other extremophile organisms is under way. Furthermore, the sensitivity to ionising radiation of the two allosteric forms and the possible protective effect of FBP and NADH against radiation damage is currently being investigated.

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Keywords: LDH, radiation damage, allostery

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Interactions of Phospholipids with Integral Membrane Proteins; Use of Brominated Lipids

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The lipid environment of integral membrane proteins is important to their structure and function, but little is known about specific protein-lipid interactions. Knowledge of these will help in devising better protocols for solubilisation and crystallisation of membrane proteins. Although some lipids may co-purify with the protein and show as residual electron density, the detergent used in solubilisation can confuse the interpretation. By co-crystallising the protein with brominated lipids their binding sites can be distinguished.

We have located two lipid binding sites in a complex of the reaction centre (RC) from *Rb. sphaeroides* with the 1-Palmitoyl-2-Stearoyl(6,7)-dibromo-sn-glycero-3-Phosphocholine (Br-PC) based on single wavelength data. We are now producing complexes of RC with variety of brominated lipids changing the lipid headgroup, the number and positions of bromine atoms, and concentration of the lipid in co-crystallisation with RC. We would also like to perform MAD experiments for some of these complexes at the Br K-edge (around 13.47 keV) to improve the anomalous signal and also to assess the possibility of using this method to phase structures of integral membrane proteins. Results of these experiments will be described.

The authors are members of the membrane protein structure initiative (MPSI), supported by the BBSRC.

Keywords: membrane protein crystallisation, brominated phospholipids, MAD and SAD experiments

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Structural Studies of Water-soluble Chlorophyll Protein from *Chenopodium album*

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Generally, chlorophyll (Chl) molecules functioning in photosynthesis are associated with hydrophobic integral membrane proteins. Water-soluble Chl protein (WSCP) was first found in *Chenopodium album* in 1963. WSCPs have then been detected in several species classified in the Polygonaceae, Chenopodiaceae, Amaranthaceae and Brassicaceae families. Although the physiological function of WSCPs has not yet been cleared, these WSCPs can be categorized into two classes according to their photoconvertibility: *Chenopodium*-type (Class I) and *Brassica*-type (Class II). The absorption spectrum of a Class I WSCP changes drastically on exposure to visible light, while a Class II WSCP does not. And there is no significant sequence homology between Classes I and II WSCPs. The X-ray structure analysis of Class II WSCPs containing *Lepidium*-, *Raphanus*- and Kale-WSCPs reveals that these WSCPs consist of 4 subunits and a Chl is contained in each subunit. In order to determine the crystal structure of a Class II WSCP and elucidate the photoconversion mechanism, *Chenopodium*-WSCP was extracted from leaves, purified, and crystallized in a dark room. Green rod crystals appeared in a week. A native data set was collected to 3.0 Å resolution at 100 K with synchrotron radiation at PF. The space group of the crystal was determined to be orthorhombic *I*222 with unit-cell parameters a = 47.08, b= 61.42, and c = 107.0 Å. Heavy atom derivative screening for structure determination is in progress. The photoconversion mechanism and the interaction between Chl and the protein are being studied.

Keywords: water-soluble chlorophyll protein, photoconvertibility, pigment protein

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Structure of the C-terminal Domain of DipZ from *Mycobacterium tuberculosis*

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DipZ (Rv2874) from *M. tuberculosis* is a member of the CcdA protein family. This family of proteins shares a conserved transmembrane electron transport domain with similarity to CcdA from *Rhodobacter capsulatus* and varies in size from 190 aa to over 750 aa. It has been proposed that the function of the larger proteins has been modified by the acquisition of extra-cytoplasmic protein domains. The transmembrane region functions by passing electrons from the cytoplasm of the cell across the membrane for use by these extra-cytoplasmic domains [1].

The C-terminal soluble domain from DipZ has been crystallised and the structure determined by SAD methods from crystals soaked in K_2PtCl_4. The model reveals a dimeric structure. Each subunit is comprised of two domains, an N-terminal thioredoxin-like fold predicted from earlier sequence alignments, and a C-terminal jelly-roll fold with similarity to the family 6 carbohydrate binding modules. A