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Names and affiliations of applicants (*indicates experimentalists):

Dr William N. Hunter
The Wellcome Trust Building
Department of Biochemistry
University of Dundee
Dundee
DD14HN
UK

Report:

Glycosyltransaferases from bacteria play key roles in the synthesis of components of the cell walls with implications for the cells antigenic properties, infectivity and pathology. They are of interest as targets for chemotherapeutic intervention and as possible catalysts in bio-transformation chemistry. Structural models are required to assist an understanding of these enzymes mechanisms and substrate specificity. To this end we have initiated crystallographic studies of two glycosyltransferases, namely the a-D-glucose - 1 -phosphate cytidyltransferase from ***Yersinia pseudotuberculosis*** (GPCT) and the a-D-glucose- 1 -phosphate thymidylyltransferase from ***Salmonella enterica*** LT2 (GPTT). Both GPCT and GPTT are functional as homotetramers of subunit molecular masses 29 kDa and 32.5 kDa respectively.

Both enzymes have been cloned and overexpressed in high yields and purified by Prof Lui's laboratory (University of Minnesota, USA) for crystallisation trials. Numerous crystals forms have been obtained for each enzyme. In each case some of the crystal forms attain a good size (up to 0.5 mm minimum dimension) and display well defined morphology. Some forms have been too small to use for diffraction experiments. Crystals were used for experiments at ESRF to ascertain quality and seek out samples for data collection. Fairly long exposures were required for many of the samples enabling us to characterise the Unit cells of several forms.

GPCT
Crystal type I
Cubic a = b = c = 243Å Primitive, space group not determined. Diffracts to 6.0Å.

Crystal type II
Hexagonal a = b = 228, c = 129Å. $P6_1$ or enantiomorph. Diffracts to 5.0Å.

GPTT
Crystal type I Tetragonal, $P4_32_12$ or enantiomorph a = b = 238.9, c = 102.9Å. Diffracts weakly to 4.0Å. Probably 2 tetramers per asymmetric unit.

Crystal type II Monoclinic $P2_1$, a = 137.6, b = 112.8, c = 181.9Å, $\beta = 97^o$. Diffracts weakly to 4.0Å. 2 or 4 tetramers per asymmetric unit.

Crystal type III Orthorhombic $P2_12_12_1$, a = 105.2, b = 177.1, c = 331.3Å. Possibly 2 to 4 tetramers per asymmetric unit. Diffraction to 5.0Å observed.

Given the high quality of the X-ray beam used for the experiments then the poor quality and limited diffraction observed with all the crystals was disappointing. It led us to conclude that the level of structural detail required to understand aspects of these enzymes structure, function and mechanism could not be attained from our samples. Studies with these particular two glycosyltransferases has now ceased. Efforts will be made with examples of these enzymes from other sources and if suitable crystals are obtained we shall apply for further time at ESRF.