MS–03.06.06 PROTEIN OLIGOSACCHARIDE INTERACTIONS: GLYCOGEN PHOSPHORYLASE AND LYSOZYME. By L. N. Johnson & A. Hadfield, Laboratory of Molecular Biophysics and Oxford Centre for Molecular Sciences, University of Oxford, Oxford, OX1 3QU, UK.

Both glycogen phosphorylase and lysozyme exhibit oligosaccharide recognition sites, for α-(1-4) and β-(1-4) linked glucosyl polymers respectively. Phosphorylase has an external glycogen storage site that allows the enzyme to bind to glycogen particles in muscle. The details of the recognition of the site have been described (Goldsmith et al. J. Mol. Biol.1982, 156, 411-427; Johnson et al. Current Topics Microbiol 1988, 139, 81-134; Goldsmith et al. Trans Am. Cryst. Soc. 1989, 25, 87-104;, Johnson et al. J. Mol. Biol. 1990, 211, 645-661). All the contacts to the oligosaccharide at the major binding site are included in the sub-domain from residues 398-437 which has α-α-β-β topology. In the X-ray studies the 5 α-(1-4) linked glucosyl sugars of maltopentaose adopt a left handed amylose-like helix so that the 2 ends curl away from the protein. The contacts are dominated by hydrogen bonds from the protein to the 2 central sugars and van der Waals contacts from non-polar residues (including a tyrosine) to the non-polar face of the sugars. Binding of monosaccharides at the catalytic site has shown that here interactions are dominated by a net work of hydrogen bonds from planar polar groups on the protein to each of the hydroxyl groups on the glucose (Martin et al. Biochemistry, 1990,29, 10745-10757; Martin et al., Biochemistry, 1991, 30,10101-10116). These interactions obey the rules formulated by Quiocho for protein carbohydrate recognition with the exception that there is only one charged group involved in the polar contacts and no aromatic residues in the van der Waals contacts. Rabbit muscle glycogen phosphorylase exhibits low affinity for oligosaccharides at the catalytic site. Examination of the R state active form of the enzyme, in which there is ready access to the site, shows that either the oligosaccharide must adopt a conformation that is different from the preferred conformation for α(1-4) linked glucosyl polymers or (and this seems less likely) the glucosyl residue in the terminal site must shift. Understanding the recognition of the oligosaccharide component of the substrate forms the major unsolved problem in understanding phosphorylase catalysis.

Recognition of oligosaccharide substrates by lysozyme has been well described following the fundamental work by David Phillips and his team in 1967. Recently we have examined binding of oligosaccharide to a mutant lysozyme, Asp52Ser, in collaboration with C. M. Dobson, G. Lowe, S. Radford & R. Aplin in Oxford. The D52S mutant, supplied by D. B. Archer, AFRC, Norwich, exhibits less than 1% activity of the native enzyme (Lumb et al. FEBS Lett. 1992, 296, 153-157) and this allows the possibility of co-crystallisation experiments in the presence of oligosaccharide substrate, $GlcNAc_6$. In our first experiment crystals took about 14 days to grow before they were analysed by X-ray crystallography and the structural results showed that catalysis had taken place. The product tetrasaccharide, $GlcNAc_4$, was bound at the catalytic site. The sugar in site D had the C1 hydroxyl in the α configuration and this hydroxyl was hydrogen bonded to Ser52 and to a water molecule. Analysis of crystals that had been obtained in a shorter time period (5 days) showed that there had been a rotation of the molecule in the lattice and analysis of the oligosaccharide bound is in progress.

The lecture will outline the important features of oligosaccharide recognition learnt from these 2 examples and describe the latest results on the catalysis in the crystal.

MS–03.06.07 MALTOTETRAOSE-COMPLEXED STRUCTURE OF AN *EXO*-TYPE MALTOTETRAOSE-FORMING α–AMYLASE FROM *P. STUTZERI*. By Y. Yoshioka, Y. Matsuura*, M. Sato, Y. Katsube, Institute for Protein Research, Osaka University, Suita, Osaka 565, Japan, and M. Kubota, Hayashibara Biochemcal Lab. Inc., Amase-minami, Okayama 700, Japan.

Maltotetraose forming α-amylase (G4-amylase) of 429 amino-acid residues from *Pseudomonas stutzeri* is an *exo*-type α-amylase which degrades starch from its non-reducing end. We have previously determined the crystal structure of this enzyme by the multiple-isomorphous replacement method at 2.2 Å resolution (Morishita et al., 1993, submitted to *J. Mol. Biol.*). Here we report the structures of the maltotetraose-complexed forms of this enzyme. The sole enzyme crystallizes in orthorhombic $P2_12_12_1$, a=65.6, b=170.5 and c=46.7 Å from 0.9 M ammonium sulfate solution. The complexed crystals were obtained using the same precipitant by co-crystallization of E219Q mutant of this enzyme with maltopentaose in two-forms: I. orthorhombic $P2_12_12_1$, a=65.1, b=138.4 and c=50.7Å, and II. orthorhombic $P2_12_12_1$, a=65.5, b=170.7 and c=46.8 Å. Intensity data to 1.9 Å resolution were collected on an imaging-plate diffractometer Rigaku RAXIS-IIc from one crystal for each form with Rmerge of 5.4% and 8.9%, respectively. The structure of the form I was determined by the molecular replacement method with the program AUTOMR (Matsuura, 1991, J. Appl. Cryst. 24, 1063-1066) by using the native enzyme as a search model, followed by a rigid-body refinement by the program CORELS at 2.5 Å resolution giving an R-factor of 31.4 %. The form II was almost isomorphous with the native uncomplexed crystal, and the coordinates in the native crystal were subjected to the rigid-body refinement to an R-factor of 28.8 % at 2.5 Å resolution. 2Fo-Fc maps for these crystals calculated at this resolution both clearly showed the bound maltotetraose instead of maltopentaose at the active cleft of the enzyme. Starting from these structures the refinements were performed by the restrained least-squares method by using the program PROFFT and the interactive graphics program FRODO. The present R-factors and resolutions of refinement are 16% at 1.9 Å resolution and 20% at 2.5 Å resolution for form I and II, respectively.

The overall structure of G4-amylase, being no large difference between complexed and uncomplexed forms, is similar to those of known *endo*-type α-amylases (Matsuura *et al.*, 1984, *J. Biochem.* 95, 697-702; Buisson et al., 1987, *EMBO J.* 6, 3909-3916). It consists of two domains; A from residues 1 to 360 and B from residues 361 to 429. Domain A has within it a $(\beta/\alpha)_8$ barrel structure and a branch region forming an active cleft between them. There are two calcium ions and two disulfide bonds in domain A. The primary calcium site with seven ligands is the one which is conserved in α-amylases. The second with six ligands is located at the bottom of the barrel near N-terminal residues. Domain B is consisted of a five-stranded anti-parallel β-sheet.

Maltotetraose bound in the active cleft takes a loose helically curved conformation with its direction of reducing and non-reducing end same as expected from the study of Taka-amylase (Matsuura et al.,1984, *J. Biochem.* 95, 697-702). The hydroxyl group (O4) of the non-reducing end glucosidic residue (Glc1) is hydrogen-bonded to Asp160-OD1 (2.77 Å) and Gly158-N (3.13 Å), which may play a key role for the recognition of the non-reducing end of substrate that determines *exo*-wise degradation. The hydroxyls O3 and O6 of Glc1 are respectively hydrogen-bonded to a water molecule and Asp160-OD2. The glucosidic residues Glc2 and Glc3 are mainly supported by the van der Waals contacts between hydrophobic and aromatic residues at their both faces. The reducing end glucosidic residues Glc4 is extensively hydrogen-bonded, i. e., ten with amino acids and two with water molecules, resulting a rotation of Glc4 with respect to Glc3 about the glucosidic linkage. The reducing-end hydroxyl group (O1) is hydrogen-bonded to the catalytic residues Glu(Gln)219-OE1 (3.21 Å) and Asp294-OD1 (3.38 Å). The most prominent structural change between complexed and uncomplexed molecules is that OD1 and OD2 of Asp294 moved toward Glc4 enabling the formation of hydrogen-bonds to its O2 and O3, respectively.

