MS-03.06.05 PRINON DIOGLACXARIDE INTERACTIONS: GLYCOGEN PHOSPHORYLAKE AND LYSOZYMME. 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 bonding site are included in the subdomain from residues 398-437 which has α-α β-β topology. In the X-ray studies the 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 motosaccharides at the catalytic site has shown that the interactions are dominated by a network of hydrogen bonds from polar and non-polar groups to 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, 10101-10111). These interactions obey the rules formulated by Quesada for the 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. Rabih muscle glycogen phosphorylase exhibits low affinity for oligosaccharides at the catalytic site. Examination of the α 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 glucose 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. Doherty, C. Lowe, S. Radford & R. Aplin in Oxford. The DYS2 mutant, supplied by D. B. Archer, AFR, Norwich, exhibits less than 1% activity of the native enzyme (Lamb et al. FEBS Lett. 1992, 296, 153-157) and this allows the possibility of co-crystallization experiments in the presence of oligosaccharide substrate, GlcNAc2. 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, GlcNAc2, 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 MALTO-TROASE-COMPLEXED STRUCTURE OF AN EVO-TYPE MALTO-TROASE FORMING α-MYELASE FROM P. STUTTERI. By Y. Yoshikawa, Y. Sato, Y. Kato, Institute for Protein Research, Osaka University, Suita, Osaka 565, Japan, and M. Kubota, Hayashibara Biochemical Lab. Inc., Amano-minami, Okayama 706, Japan.

Maltooligos acforming 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 (Murata et al., 1993, submitted to J. Mol. Biol.). Here we report the structures of the maltooligosaccharide-complexed forms of this enzyme. The so-called enzymes is as orthorhombic P21_21_21, a=65.6, b=170.3, c=46.7 Å from a 0.9 M ammonium sulfate solution. The complexed crystals were obtained using the same precipitant by co-crystallization of a 219Q mutant of this enzyme with maltopentaose in two forms: I orthorhombic P21_2_2, a=65.1, b=138.4, c=50.7Å, and II orthorhombic P21_21_2, a=65.5, b=170.7, c=46.8 Å. Intensity data to 1.9 Å resolution were collected on an imaging plate diffractometer Rigaku RAXIS-IV from one crystal for each form with ResNeXt of 3.45° and 3.56°, respectively. The structure of the form I was determined by the molecular replacement method with the program AUTOMR (Matsura, 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 CRONAL 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 28.9% at 2.5 Å resolution. 2Fo-Fc maps for these crystals calculated at this resolution both clearly showed the bound maltotetraose instead of maltobiose at the α,β,ε clef of the enzyme. Starting from these structures the refinement were performed by the restrained least-squares method by using the program PROFFIT and the interactive graphics program FRODO. The present R-factors and resolutions of refinement are 16% at 1.3 Å resolution and 23% at 2.5 Å resolution for form I and II, respectively.

The overall structure of G4-amylase is not as large change between complexed and uncomplexed forms, is similar to those of α-myelase type α-amylases (Matsura et al., 1984, J. Biochem. 95, 697-712; Buusen 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 β/β/β 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 composed of a 5 stranded anti-parallel β-sheet. Maltooligosaccharide 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 (Matsura et al., 1984, J. Biochem. 95, 697-712). The hydroxyl group of Glc of the non-reducing end glucosidic residue (Glc-I) is hydrogen-bonded to Asp160 (OD1 12.27 Å and Gly158 N 1.13 Å), which may play a key role for the recognition of the non-reducing end of substrate that determines exo-wise degradation. The hydroxyls of 01 and 02 of Glc-I are respectively hydrogen-bonded to a water molecule and Asp160 (OD2 3.06 Å and Asp34 3.38 Å). The most prominent structural change between complexed and uncomplexed molecules is that OD1 and OD2 of Asp34 moved toward Glc-I enabling the formation of hydrogen-bonds to its O2 and O3, respectively.