02.1-35 X-RAY STUDIES OF ACTIVATION/CATALYSIS IN GLYCOGEN PHOSPHORYLASE a. By S.R. Sprang and R.J. Fletterick, Dept. of Biochemistry & Biophysics, University of California, San Francisco, CA 94143.

The atomic coordinates of the glucose inhibited, T, conformer of glycogen phosphorylase a (pa) have been refined by a constrained least-squares procedure with diffractometer data at 2.1 Å resolution. We report the active site conformation of the refined T structure, and the conformation changes concomitant with the binding of substrate analogs.

Catalysis requires a ternary complex of the active, R, pa conformer with an α(1→4) linked oligosaccharide chain of glycogen, and inorganic phosphate (Pi). Only binary active site complexes with substrate analogs, viz. phosphite (PO_3^{-2}) or α-D-1,2 cyclic glucose phosphate (GCP) can be investigated cyrstallographically by difference Fourier analysis. These reveal significant elements of the T→R transition. Glucose bound T pa models the interaction of the non-reducing end of glycogen with the enzyme. Involvement of all hydrogen bonding sites of the glucose molecule rationalizes the specificity of pa for α-D-glucosides. Glucose stabilizes a T conformation in which it and the active site residues are inaccessible to solvent. The Pi binding site is occluded by the salt bridged (to R568) D283 residue located in a β-turn segment at the molecular surface. Binding of Pi or GCP results in dislocation of this segment and reveals a phosphate binding site containing R568, K573 and H570. The active site residues are now solvent accessible. Model building studies locate the glycogen fragment at a catalytically strategic position in a preferred conformation.

The postulated ternary complex suggests a mechanism in which glycosidic oxygen is protonated by Pi, assisted by H570 as acid catalyst. The coenzyme pyridoxal phosphate likely assists in the proton transfer and in the nucleophilic attack by Pi on the resulting carbonium ion.

02.1-36 GLYCOGEN PHOSPHORYLASE b: STRUCTURE DETERMINATION AND REFINEMENT. By D.I. Stuart, K.S. Wilson, E.A. Stura, D.L. Wild, J.A. Jenkins and L.N. Johnson, Laboratory of Molecular Biophysics, South Parks Road, Oxford OX1 3PS.

Glycogen phosphorylase catalyses the first step in glycogen degradation releasing glucose-1-phosphate from the non-reducing end. Phosphorylase b, the form found in resting muscle, requires AMP or IMP for activity and is inhibited by ATP and glucose-6-phosphate.

Large single crystals of phosphorylase b, grown in the presence of the weak activator IMP, are tetragonal space group $P4_32_12$ with unit cell dimensions a = b = 128.5Å, c = 115.9Å and one subunit (MW 97,333) per asymmetric unit. The crystal structure has been solved at 3Å resolution from an electron density map with phases calculated from contributions of 4 heavy atom derivatives (m = 0.64). A molecular model (scale 2cm/Å) has been built for almost all the 841 amino acids. The co-ordinates for 6519 atoms have been refined by the constrained least squares method of Konnert and Hendrickson.

Data at 2Å resolution are too weak and too radiation sensitive to record on oscillation photographs with conventional X-ray sources. These data have been successfully collected using the intense electron synchrotron source at LURE, Orsay. An approximate 5-fold improvement in resistance to radiation damage was noted. The resolution of the structure has been extended to 2.5Å by incorporation of these data into the refinement. The current R value from the refinement (which is still in progress) is 0.38 at 2.5Å resolution with a r.m.s. deviation from standard bond distances of 0.06Å.

A precise description of the molecular structure awaits completion of the refinement but the fold of this large molecule is already clear. It can be described in terms of 3 domains. The N-terminal domain comprising the first 312 residues; the glycogen binding domain (residues 322-485); and the C-terminal domain (residues 485-841). The overall shape of the subunit is compact except for 2 loops, the cap (residues 36-45) and the tower (residues 251-277) which extend into the symmetry related molecule of the physiologically active dimer. The molecule contains extensive secondary structure. In particular at the centre of the C-terminal domain is a β/α region of 6 parallel strands and 5 α-helices with identical topology to the nucleotide binding domain of lactate dehydrogenase and related enzymes. The essential co-factor, pyridoxal phosphate, is linked via a Schiff base to Lys 679 from the α-E helix of this domain and is buried in the centre of the molecule. The conformation for the torsion angle C4.C5.C5'.O5' is trans and is similar to that observed in single crystal structures of pyridoxal phosphate. The phosphate moiety is stabilized in this buried environment by interactions with the helix dipole of αE and the ε-amino group of Lys 567 from the βA strand.

The first 19 residues of phosphorylase b cannot be located in the electron density map and it is assumed that they are mobile consistent with solution studies. The crystal structure shows that these residues cannot be involved directly at the allosteric effector site. Nevertheless it has been shown by the work of Graves et al. on phosphorylase b' (the limited trypsin modified enzyme comprising residues 17-841) that the N-terminal peptide is important for the heterotropic control properties of phosphorylase b.

Several lines of evidence suggest that while crystals of phosphorylase b in the presence of IMP are active, the conformation of the molecule in the crystal is close to the T state, which has a low affinity for substrate. Attempts to obtain crystals of the R state are in progress.

02.1-37 GLYCOGEN PHOSPHORYLASE b: CONTROL AND ACTIVITY. By K.S. Wilson, E.A. Stura, J.A. Jenkins, M.S.P. Sansom, D.I. Stuart and L.N. Johnson, Laboratory of Molecular Biophysics, South Parks Road, Oxford OX1 3PS.

Extensive metabolite binding studies on glycogen phosphorylase b in the crystal have been carried out at 3Å resolution. Four important sites have been recognized.

(i) The catalytic site: This is situated in the interior of the molecule where the 3 domains come together and is accessible to solvent through a narrow channel. The site is close to the essential co-factor pyridoxal phosphate. The substrate glucose-1-phosphate and the potent inhibitor glucose-1,2-cyclic phosphate bind tightly at this site with no disruption of the crystal lattice. The results indicate that binding is determined mostly by the glycosyl portion and that the phosphate binding site is weak in the T state of the enzyme.

(ii) The allosteric effector site: This is some 32Å away from the catalytic site in the N-terminal domain and at the subunit-subunit interface of the dimer. The activators AMP, IMP and inorganic phosphate and inhibitors ATP, G6P all bind at this locus. The strong activator AMP and the strong inhibitor G6P tend to disrupt the crystal lattice indicating that both these metabolites cause conformational changes that are in conflict with the crystal lattice forces. In contrast the weak effectors IMP and ATP bind with no disruption and no conformational changes. The phosphate moieties of AMP and G6P occupy two different but mutually exclusive sites separated by 2.2Å while the adenine-ribose portion of AMP and the glucose of G6P occupy different and non-overlapping sites.

(iii) The glycogen binding site: All oligosaccharides and glycogen analogues studied so far bind to this site on the surface of the enzyme which is some 30Å from the

catalytic site and 39Å from the allosteric site. Oligosaccharides bind in the preferred conformation for α(1-4) linked glycopyranosyl polymers in which there is an internal hydrogen bond between the O2 and O3 hydroxyls of adjacent sugars. The major interaction is with 4 turns of the long α-helix (residues 396-416) and illustrates an interesting complementarity between the secondary structures of these two different polymers. (iv) The nucleotide/nucleoside inhibitor site: This is some 12Å from the catalytic site and provides a second binding site for adenine nucleotides, nucleosides and caffeine.

Crystallographic evidence suggests all these sites interact with one another. For example binding of oligosaccharide tightens the binding of nucleotide at the allosteric site but, in the crystal, weakens the binding of G1P at the catalytic site.

Proposals for the essential features of a catalytic mechanism have been put forward from observations on the proximity of the 5'-phosphate group of pyridoxal phosphate to substrate, G1P, combined with model building studies (Johnson et al., J. Mol. Biol. 140, 565-580). In order to reconcile the X-ray evidence with known biochemistry and stereochemistry it was proposed that the substrate binding to the T state of the enzyme represents a non-productive binding mode and that when the enzyme converts to the high affinity R state in the presence of AMP and glycogen an alternative binding mode is adopted. In the proposed mechanism the 5'-phosphate group of the pyridoxal phosphate functions as a nucleophile to stabilize a carbonium-oxonium ion intermediate and a histidine, His 376, functions as a general acid to donate a proton to the glycosidic oxygen. The results obtained with glucose-1,2-cyclic phosphate (Jenkins et al., Proc. Roy. Soc. B (1981) in Press) are compatible with these proposals but further studies are required in order to test the hypothesis.

02.1-38 THE STRUCTURE OF GLUCOSE-6-PHOSPHATE ISOMERASE AT 2.6 Å RESOLUTION. A. Achari, S.E. Marshall, H. Muirhead and P.J. Shaw*. Dept. of Biochemistry, University of Bristol, Bristol, BS8 1TD., U.K.

Glucose-6-phosphate isomerase (E.C.5.3.1.9) is the glycolytic enzyme responsible for the interconversion of D-glucose-6-phosphate and D-fructose-6-phosphate. The enzyme from porcine skeletal muscle crystallizes from ammonium sulphate solution in space group $P4_32_12$, with a = 95.2 Å, c = 138.3 Å. The data for 2.6Å resolution were collected on a rotation-oscillation camera. An electron density map at a nominal resolution of 2.6 Å has been calculated using phases obtained from isomorphous and anomalous data. Phases between 3.5 Å and 2.6 Å resolution were calculated with data from a mercury derivative. The enzyme contains two identical subunits related by a diad axis. Two distinct domains in each subunit have the α/β type structure (Shaw, P.J. and Muirhead, H., J. Mol. Biol. 109, 475-485, 1977).

Sequence information is available for about one third of the molecule from five cyanogen bromide peptides (E.A. Noltmann, University of California, Riverside, U.S.A., private communication). A tentative fit of two of these peptides has been made. One of these peptides has a reactive cysteine which has been labelled chemically as a carboxymethyl residue and crystallographically as a mercurial derivative.

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02.1-39 RIBONUCLEASE-A : LEAST-SQUARES REFINEMENT OF THE STRUCTURE AT 1.45Å RESOLUTION. By Nivedita Borkakoti, David S. Moss and Rex A. Palmer, Department of Crystallography, Birkbeck College, Malet Street, London, UK.

The crystal structure of bovine pancreatic ribonuclease-A has been refined by restrained least squares analysis employing X-ray diffractometer data to 1.45Å resolution. The current R-factor for 19,238 reflections is 0.26. The rms deviation from ideality of bond lengths is 0.01Å. Minor corrections to previously reported features of secondary structure are presented and a quantitative analysis of intra molecular hydrogen bonds is given. A total of 79 water or ethanol solvent molecules in the first coordination sphere around the enzyme molecule have been clearly identified and included in the least-squares analysis. A sulphate anion, characterized chemically by laser Raman spectroscopy, and prominently displayed in the 1.45Å electron density has also been refined as part of the structure. Further new features of the structure to emerge are alternative positions for the active site H1S-119 side group which have been refined independently with individual occupation factors, a solvent molecule hydrogen bonded to the N-terminal amino group, and disorder of the side chains in the regions of residues 17 to 24 and 35 to 39 neither of which have any structural or biochemical significance for the molecule. Extensive model building by computer graphics has facilitated the production of a sound, accurate model of the ribonuclease-A structure which will now enable detailed and precise analysis of the catalytic mechanism to be undertaken.

02.1-40 THE STRUCTURES OF APO AND HOLO CYTOPLASMIC MALATE DEHYDROGENASE AT HIGH RESOLUTION. By J.J. Birktoft L.J. Banaszak and T. Meininger. Department of Biological Chemistry, Washington University School of Medicine St. Louis, Missouri 63110, U.S.A.

Cytoplasmic malate dehydrogenase (sMDH) crystallizes in one of two nearly isomorphous forms. The appearance of the different unit cells is determined by the presence of the coenzyme NAD and they are thought to contain sMDH in the apo and holo conformational states. A structural comparison at low resolution (5.0 Å) showed that although the overall folding of apo and holo sMDH are rather similar, substantial conformational differences do exist. (Weininger, Birktoft and Banaszak (1977) in "Pyridine Nucleotide-Dependent Dehydrogenases", ed. H. Sund, Walter de Gruyter & Co., p. 87).

In order to further understand these conformational differences as well as to obtain more accurate models, additional diffraction data extending to high resolution have been collected, and constrained crystallographic refinement initiated. Data for holo sMDH at two levels of NAD saturation (∿ 60% and 100%) were collected previously by diffractometry, as were the low resolution apo sMDH data. High resolution data, extending to 2.5 Å resolution, for apo sMDH were collected by oscillation photography. A Supper oscillation camera controlled by a microprocessor built and programmed by Drs. R.W. Wrenn and P.H. Bethge were used. The "SCAN12" program package as modified by Dr. R.M. Sweet and ourselves for the PDP 11/34-Optronics P-1000 system were used for the processing of the film data.

The two sets of holo sMDH data (60% holo sMDH and 100% holo sMDH) are to be refined using the Hendrickson-Konnert constrained refinement method. In the absence of a chemically determined amino acid sequence for sMDH, a