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.

* Current address: John Innes Institute
Norwich
Norfolk, U.K.

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

tentative "X-ray sequence" has been derived from the 60% holo sMDH electron density map. The detailed map interpretation was performed using both a "Richards Box" as well as the MMS-X graphics system. Additionally several peptide fragments as sequenced by Bradshaw and coworkers have been aligned with this "X-ray sequence". This sequence alignment was facilitated by the use of a diagonal matching technique, similar to that proposed by McLachlan for amino acid sequence comparisons. It is estimated that approximately 40% of the amino acid residues in sMDH are correctly assigned at this time.

The original MIR phases were derived for the lattice of the partially substituted holo sMDH. Since apo and holo sMDH are sufficiently isomorphous at low resolution, the phases for holo sMDH can be used for the apo form (Holo: a=139.2, b=86.6, c=58.8; Apo:a=140.7, b=87.1, c=58.8; space group for both $P2_12_12$). An initial assessment of the structural differences between the two forms have been made at low resolution, and these differences along with absence of NAD will be incorporated into the initial phase calculations for apo sMDH. Through the analysis of successive F_o-F_c and $2F_o$-F_c maps, followed by structural adjustments and recalculation of phases and structure factors a high resolution electron density map for this form for the sMDH should finally emerge. This will then provide the starting point for constrained crystallographic refinement of apo sMDH. Progress on this work will be reported and if completed, the molecular structure of apo sMDH will be compared with that of the holo enzyme.

The contributions of Dr. M. Weininger in the collection of low resolution apo sMDH data is gratefully acknowledged. (Supported by NSF Research Grant PCM-792187).

02.1-41 CONFORMATIONAL CHANGES OF ALCOHOL DEHYDROGENASE. By Hans Eklund and Alwyn Jones, Department of Chemistry and Molecular Biology, Swedish University of Agricultural Sciences, S-750 07 Uppsala 7, Sweden.

Alcohol Dehydrogenase crystallizes in two different crystal forms in the presence and absence of the coenzyme NAD. The orthorhombic apoenzyme structure has been refined to an R-factor below 20% at 2.4 Å resolution using different refinement procedures, with group temperature factor refinement as the last step. The triclinic ternary complex structure has been independently refined by constrained-restrained least squares refinement combined with graphic display modelbuilding to an R-factor of about 25% at 2.9 Å resolution.

The results of a detailed comparison between the two structures confirm the earlier presented evidence that the main conformational change is a rigid body rotation of the two catalytic domains coupled to a change of a loop in the coenzyme binding domain. Many details of the conformational changes can now be added and described with high confidence.

Phases calculated from the refined coordinates have been used to study substrate and inhibitor binding.

We shall also discuss the benefits of carrying out the crystallographic refinement of a large protein molecule (750 amino-acid residues) at medium resolution.

02.1-42 THE MOLECULAR STRUCTURE OF L-3-HYDROXYACYL COENZYME A DEHYDROGENASE. By Hazel M. Holden and Leonard J. Banaszak, Department of Biological Chemistry, Washington University, St. Louis, MO 63110.

L-3-hydroxyacyl Coenzyme A dehydrogenase (βHADH) is a mitochondrial enzyme involved in the β-oxidation of long chain fatty acids. It is of special structural interest because its catalytic reaction involves binding sites for both CoA and NAD^+-NADH. Furthermore, the enzyme may have a substrate binding site that accommodates the long hydrocarbon chain of the acyl CoA substrate, the length of which varies as the substrate shuttles repeatedly through the β-oxidation cycle.

Isolated from pig heart, βHADH is known to be a dimer of molecular weight 67,000. The amino acid sequence has recently been established and while a small region in the active site contains several homologous residues, overall there appears to be little homology to other dehydrogenases (Bitar, et al FEBS Letters, (1980), 116, 196). An early monoclinic crystal form obtained from ammonium sulfate was extremely sensitive to unit cell and space group changes in the presence of heavy atom compounds. Crystals obtained from polyethylene glycol in the presence of 0.01 M Tris, pH 8.0, however, proved suitable for an x-ray analysis. These crystals belong to the space group $C222_1$ with a=226.5 Å, b=82.2 Å, and c=124.6 Å. Three isomorphous derivatives K_2PtCl_6, CH_3HgCl and $IrCl_3$ have been prepared using crystals first soaked in an NAD^+-containing solution. Again, it was not possible to prepare isomorphous heavy atom derivatives of the apo-enzyme, results that resemble experiences with the earlier monoclinic form. The positions of the heavy atom sites were determined by inspection of the Patterson maps and confirmed by cross difference Fourier maps. After phase refinement, electron density maps at 7.1 Å resolution (3 derivatives) and 5.25 Å resolution (2 derivatives) were calculated. The envelopes of the two dimers contained within the asymmetric unit were identified in the low resolution map. The local two-fold symmetry of the βHADH dimer does not seem to be expressed in the binding sites of all of the heavy atom derivatives. However, the results of the rotation function studies and inspection of the electron density maps has led to a tentative solution for the positions of the local two-fold rotation axes.

A difference Fourier map between the crystalline apo- and holo-enzyme has also been calculated and indicates the binding of 2 NAD^+ molecules per asymmetric unit or one site per dimer. The one site per dimer stoichiometry correlates with some of the heavy atom results. By measuring fluorescence enhancement, equilibrium studies of the binding of NADH to βHADH shows that the coenzyme binding sites in each subunit of the dimer are equivalent (McLoughlin et al, unpublished results). Hence, the one site binding of the NAD observed by X-ray studies is likely to be the result of crystal packing effects. Crystals of βHADH have also been soaked in S-acetoacetylpantetheine. Although three-dimensional X-ray data has not yet been measured, preliminary precession photographs suggest that in the presence of S-acetoacetylpantetheine, the NAD^+ is no longer bound.

Progress on obtaining a high resolution map as well as the methods used to obtain the refined positions of the heavy atom sites, local dyads, and the substrate binding sites will be presented.

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