02.1–21 CRYSTALLOGRAPHIC STUDIES ON HUMAN PLASMA holo- AND apo-RETINOL BINDING PROTEIN

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Retinol binding protein (RBP) is the specific carrier of vitamin A in plasma. This well characterized molecule is the object of considerable interest because it participates in a protein-protein interaction with specific membrane receptors and prealbumin, in addition to its interaction with retinol and other retinol-analogues. Human-plasma RBP is a monomer of molecular weight 20,600 (182 amino acids) which contains a single binding site for the vitamin. We have crystallized both holo- and apo-RBP in isomorphous crystal forms (space group R3, unit cell parameters a=b=104.2 Å, c=74.5 Å, two molecules per asymmetric unit). Using the data of the native holoprotein and two heavy-atom derivatives, electron density maps at 3.0 Å resolution have been calculated. The two molecules in the crystallographic asymmetric unit are disposed as dimers approximately parallel to the two-fold axis and are exentially composed of beta structure. A low-resolution Fourier-difference map between the holo- and apo-molecule shows clearly the position of the retinol. High resolution data collection of both forms is in progress, in order to allow detailed studies of the changes induced by retinol binding to the protein.

02.1–22 STRUCTURE DETERMINATION OF THE ALLOSTERIC L-LACTATE DEHYDROGENASE FROM LACTOBACILLUS CASEI AT 3 Å RESOLUTION.

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The allosteric LDH from L.casei has been crystallized as a complex with its activators Fru-1,6-P_2 and Co^{2+}. The tetrameric enzyme crystallizes in space group C 2 with 6 tetramers in the unit cell. The overall arrangement is close to the supergroup P 3_121, all tetramers have good local 2 2 2-symmetry.

The structure was solved by Molecular Replacement using dogfish muscle apo-LDH and the ternary complex of pig heart LDH as models. In the first stage, $2F_o-F_c$ electron density of all 6 crystallographically independent subunits was averaged. "Chopped $2F_o-F_c$" maps were used for critical parts of the molecule.

The fold of the polypeptide chain is very similar to the standard LDH fold, with some notable exceptions: The first 12 amino acids are lacking, L.casei LDH does not have an N-terminal arm; the fold of the active site loop is different from all known (apo or ternary) conformations; amino acids 209 – 224 appear not well ordered in an averaged chopped map; the C-terminus is extended by 2 turns of helix.

In the active center, an extended relay system of hydrogen bonds is clearly visible. It connects a sulfate ion (which occupies the substrate position) with His 195, Asp 168 and Glu 199 linearly, finally reaching the solvent.

The activator Fru-1,6-P_2 binds to an extended site not far from the molecular P-axis. One phosphate subsite is formed by arginines 173 and 185 and His 188 (this subsite is an unspecific anion binding site in other LDHes) and the other phosphate subsite involves Arg 256 and, possibly, Lys 259. Hydrogen bonds from the sugar extend to Tyr 190 and to the helix which carries the essential residues Thr 165, Asp 168 and Arg 171.

02.1–23 CRYSTAL STRUCTURE OF THE CALF EYE LENS PROTEIN GAMMA-CRYSTALLIN III.

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A main calf lens protein, the gamma-crystallin fraction IIIb with a molecular mass ≈ 20.0 kDal was crystallized in space group $P2_12_12_1$ with two molecules in the asymmetric part of the unit cell. The phases were obtained at 3.0 Å resolution with m = 0.72 by isomorphous replacement and anomalous dispersion with five derivatives. These phases were expanded and refined by an improved version of the method of Agarwall & Isaacs (Proc. Natl. Acad. Sci. USA (1977), 74, 2835) up to 2.7 Å resolution, a limit of crystal diffraction. The preliminary atomic model of gamma-crystallin IIIb consists of about 75% side chains which have been localized during the refinement process. The polypeptide chain forms four-repeated β-structural motifs packed in two similar domains. The majority of functionally important cycteine residues are in the N-terminal domain. Most of them are located inside the domain, though very close to its surface. The main chain folding of gamma-crystallin IIIb is very similar to that of gamma-crystallin II (Wistow et al., J. Mol. Biol. (1983), 170, 175) which has a homology in the amino acid sequence of about 80%. The main difference between the two crystal structures pertains to the crystal packing and intermolecular surface-to-surface interactions.

02.1–24 THE STRUCTURE OF M-TYPE PYRUVATE KINASE.

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Pyruvate Kinase is a tetrameric enzyme which catalyses the transfer of a phosphoryl group from phosphoenol pyruvate (PEP) to ADP in the presence of both bivalent and monovalent cations. The regulation of its activity is important in the control of glycolysis, especially in tissues capable of gluconeogenesis, and several classes of isoenzymes have been identified. The L-type enzyme shows a sigmoidal dependence of activity on PEP concentration and its activity is controlled by various allosteric effectors and inhibitors. In contrast the M-type enzyme does not normally possess allosteric properties. The available evidence suggests that all isoenzymes of pyruvate kinase have essentially the same tertiary structure, which can take up one of several closely related conformations. Normally in vivo the M-type enzyme is in the fully active conformation, while the exact conformation of the other isoenzymes will depend upon the concentration of substrates and the various allosteric activators and inhibitors. The tetrameric quaternary structure may well vary.

The M-type enzyme from cat muscle crystallizes in the orthorhombic space group I222 with unit cell dimensions of a = 88.4Å, b = 115.3Å and c = 131.0Å and a single subunit in the crystallographic asymmetric unit. The amino-acid sequence is known for the chicken muscle, cat

muscle and yeast enzymes. A model of one subunit was built using the computer graphics program Bilder designed and written by R. Diamond. The model comprises a short N-terminal peptide plus three domains A, B and C containing a total of 515 residues compared with the 529 contained in the sequence. There appears to be no electron density for either the N-terminal or the C-terminal residues. This model was used as the starting point for a restrained parameter, least squares refinement using the Konnert program implemented on the S.E.R.C. Cray-1 computer. The final crystallographic R-factor for the 2.6Å data was 0.283 and the final structure deviated from ideal bond lengths by an overall root-mean-square deviation of 0.019Å.

The active site has been located by studying the binding of bivalent cations, PEP and ATP. The overall homology between the muscle and yeast sequences is about 42% and side-chains close to the active site are highly conserved. Results of model-building experiments will be described.

02.1–25 MOLECULAR MODELLING APPROACHES TO THE CORRELATION OF STRUCTURE AND SOLUTION PROPERTIES OF THE SERINE PROTEASES. By T. F. Kumosinski[1], E. M. Brown[1], and M. N. Liebman[2]. [1]Eastern Regional Research Center, USDA, Philadelphia, PA 19118, and [2]Mt. Sinai School of Medicine, SUNY, N. Y., NY 10029, USA.

Serine proteases are responsible for a variety of biological control processes, such as fertilization and complement activation, via so-called limited proteolysis. These control processes involve exact macromolecular recognition. We have initiated studies of this recognition using calculations based on the three-dimensional X-ray crystallographic coordinates in conjunction with solution physical chemical methods. The modelling techniques used consisted in topological analysis (electrostatic potential surface mappings, partioned distance matrix analysis, van der Waals and accessible surface mappings) and a new internal energy profile analysis (dipole moment alignments, hydrophobicity indices, dipole-dipole, charge-dipole, and charge-charge interactions in a matrix representation). Sedimentation equilibrium and circular dichroism analyses on both enzyme and macromolecular inhibitor were performed to determine the free energy change on complex formation (ΔG_c) and the extent of autoproteolysis, as well as the influence of Ca binding and pH on ΔG_c and the regulation of autoproteolysis. The results for a number of serine proteases verified the existence of eight soft β-turns distributed on either side of the active site (MNL). They suggest point-site mutations which could modify the specificity of these natural protease inhibitors. (MNL is the recipient of a Revson Foundation Fellowship.)

02.1–26 CRYSTALLOGRAPHIC STUDIES OF BLUE PROTEIN FROM A. FAECALIS AT 2.8Å. E.T. Adman, Dept. of Biological Structure, Univ. of Washington, Seattle, Wash. 98115 and T. Beppu and H. Watanabe, Dept. of Agricultural Chemistry, Univ. of Tokyo, Bunkyo-ku, Tokyo 113

The blue protein from Alcaligenes faecalis strain S-6 is a Type I blue copper protein (cupredoxin) which is specifically required for transfer of electrons to a copper containing nitrite reductase, both in the normal electron transfer pathway whereby nitrates are converted to N_2, and in an abortive pathway whereby the reductase is inactivated in the presence of O_2.

Partial sequence information, the amino acid composition, and the molecular weight (intermediate to the two other blue copper proteins with known structure, plastocyanin and azurin) suggest that while A. faecalis blue protein probably has the same chromophore, it may have different folding, and may well represent a structural subclass of these cupredoxins.

This cupredoxin crystallizes in space group $P6_{5}$ with cell dimensions a = b = 49.9Å, c = 98.4Å, γ = 120°. The ratio of cell volume to molecular weight is 2.95, consistent with one 12,000 unit in the asymmetric unit with 60% solvent. Native diffraction data has been collected on a Picker FACS-1 diffractometer to 2.8Å resolution; data from a uranyl nitrate soaked crystal has also been collected to 2.8Å. The Patterson indicates one major heavy atom site.

Progress in determination of this structure will be reported. Support for ETA from NIH grant GM13666 is gratefully acknowledged.

02.1–27 X-RAY CRYSTALLOGRAPHY OF FAB FRAGMENTS OF ANTI-DIGOXIN AND ANTI-ARSONATE ANTIBODIES. D.R.Rose[1], G.A.Petsko[1], M.Margolies[2], J.Novotny[2], E.Haber[2] and M.Gefter[3], Depts. of [1]Chemistry and [3]Biology, M.I.T., and [2]Mass. General Hospital, Boston, Mass., U.S.A.

Crystals have been obtained of Fab fragments of monoclonal antibodies to the cardiac glycoside, digoxin. The Fab's are of clinical use in reversing the toxic effects of digoxin overdose[4]. Preliminary data on crystals of Fab 26-10 grown from mixtures of MPD and PEG(6000) will be presented.

Crystals of three anti-phenylarsonate hybridoma Fabs have been grown. Two(36-71 and 36-65) display the major idiotype of anti-arsonate antibodies in the A/J strain mouse and one (36-60) displays a minor idiotype. Data have been taken from Fab 36-71 crystals ($P2_1$, a=65.1, b=73.2, c=45.2Å, β=104.6°, grown from PEG(6000) at 20°C). Fab 36-60 crystallized at 4°C from PEG(6000) solutions in tetragonal space group $P4_32_12$ or enantiomorph with cell dimensions 68.6, 68.6, 247.0Å. A report on the progress of the structure solutions will be presented.

Abbreviations: MPD, 2-methyl-2,4-pentanediol, PEG, Polyethylene Glycol

[4]Margolies, M., Mudgett-Hunter, M., T. Smith, J. Novotny, and E. Haber (1981) in: Monoclonal Antibodies and T Cell Hybridomas (Hammerling, Hammerling, Kearney, eds.), Elsevier/North Holland, New York, 367-374.