02.X-7 STRUCTURAL BASIS OF THE ACTION OF THERMOLYSIN AND THE ZINC PROTEASES. Hazel M. Holden, Dale E. Tronrud, Arthur F. Monzingo, Larry H. Weaver and Brian W. Matthews, Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403 U.S.A.

High resolution X-ray crystallography has been used to determine the modes of binding to thermolysin of a series of different inhibitors including dipeptides, mercaptans, hydroxamates, N-carboxymethyl peptides and phosphonamidates. The interactions displayed by such inhibitors illustrate interactions that are presumed to occur between the enzyme and its substrates during catalysis. The crystallograhic analyses, together with model building, suggest a detailed stereochemical mechanism of action for thermolysin and, by analogy other zinc proteases such as carboxypeptidase A and the angiotensin converting enzyme. Recent analyses of a series of phosphonamidates, which are presumed to be transition-state analogues, has shown that chemically similar inhibitors can adopt dissimilar modes of binding. These different configurations provide a rationalization for large differences in the kinetics of binding that are observed for these inhibitors. Comparison of matched pairs of inhibitors allows the contribution of a single interaction (e.g. a hydrogen bond) to be determined.

02.X-8 MOLECULAR DYNAMICS AS A TOOL IN CALCULATING BINDING ENERGIES. By Paul A. Bash, Chemistry Department, Harvard University, Cambridge, MA 02138, U.S.A.

Recent access to the latest supercomputers is providing the means to develop computational methods, based on rigorous principles from quantum and statistical mechanics, that can be applied to macromolecular systems. Such methods are capable of calculating free energy and structural changes in real systems where the three-dimensional structure of the macromolecule is known in atomic detail. The development of methods that combine classical molecular dynamics, ab initio and semi-empirical quantum mechanics, and free energy perturbation techniques will be presented. Their utility will be demonstrated by calculating solvation free energies of biological molecules in solution, relative binding free energies of ligands to proteins, and the energetic pathway of an enzyme reaction.

02.X-9 PREDICTION OF THE CONFORMATION OF SHORT SEGMENTS OF POLYPEPTIDE CHAIN IN PROTEINS by J.Moult and M.N.G.James, Medical Research Council of Canada Group in Protein Structure and Function, Biochemistry Department, University of Alberta, Edmonton, Alberta T6G 2H7, Canada.

The design of segments of protein structure, the modelling of homologous proteins, and the determination of protein structure using molecular replacement methods all require an ability to predict the conformation adopted by short stretches of polypeptide chain in the presence of the rest of the structure. We report here the current state of development of a computer algorithm with such predictive power (J.Moult and M.N.G.James, PROTEINS 1 146-163 (1986)). The success of the method depends upon extracting from known protein structures a set of rules which can be used to restrict the number of conformations a chain may have, and to choose one close to the correct structure. Useful rules are the preferred values of backbone and side chain dihedral angles, the avoidance of short interatomic contacts, the optimization of electrostatic interactions, and the minimization of solvent exposed hydrophobic area. Tests of the method, simulating homologous modelling problems, show that for stretches of chain up to six residues long it is possible to identify amongst the generated conformations one with about 1Å root mean square deviation (on all non-hydrogen atoms) from the correct structure.

02.X-10 ANTIBODY-ANTIGEN RECOGNITION: THE STRUCTURE OF A FAB-LYSOZYME COMPLEX. By A.G.Amit, *R.A.Mariuzza, +S.E.V.Phillips and R.J. Poljak, Immunologie Structurale Institut Pasteur, Paris, France, *MRC Laboratory of Molecular Biology, Cambridge, UK, and +Astbury Department of Biophysics, University of Leeds, Leeds,UK.

The initial step in the activation of the immune system is the binding of foreign antigens to the surface of B and T lymphocytes, the receptor molecule on B lymphocytes being membrane bound immunoglobulin. A B lymphocyte also secretes soluble immunoglobulin molecules, or antibodies, with antigen binding domains of a single antigen specificity identical to that of its membrane bound receptors. Mild proteolysis of antibody molecules liberates these domains as antigen binding fragments (Fab) which may be purified and crystallized. A Fab is highly specific in binding its particular antigen molecule, and not others, and can even distinguish point mutations where the antigen is a protein molecule. This molecular recognition forms the basis of the specificity of the immune response, and can be studied directly in crystal structures of antibody-antigen complexes.

The crystal structure of the complex between hen egg lysozyme and the Fab fragment of a monoclonal antibody (D1.3) raised against it in Balb/c mice has been determined at 2.8Å resolution (1). Crystals of the complex grown from 15-20% PEG 6000 at pH 6.0 are monoclinic, space group $P2_1$ with a=55.6, b=143.4, c=49.1Å, ß=120.5°, Z=2. X-ray intensities for native and three heavy-atom derivative crystals were collected on a 4-circle diffractometer. The MIR electron density map (<m> = 0.47 for 15592 reflextions) was difficult to interpret, but was greatly improved by the application of a simple density modification technique. The current model has been refined to R=0.27 for all reflexions in

the 20-2.8Å resolution range. No attempt has been made to locate solvent molecules. The antibody-antigen interface extends over a large area (∿20 x 30Å), involving 17 residues in all 6 CDR loops of the Fab. Residues 18-27 and 116-129 of lysozyme contribute to the interaction, and the antigenic site is therefore "topographic" (discontinuous), owing its existence to the three-dimensional structure of the native folded protein. The interface consists of interlocking complementary hydrophobic surfaces, with specific hydrogen bonding interactions between main and side chain groups of both molecules. Specifically, Gln 121 of lysozyme lies in a hydrophobic pocket of the Fab, its side-chain amide group forming a buried hydrogen bond to the main chain carbonyl of one of the CDR loops. Mutation of Gln→His 121 in some avian lysozymes disturbs this interaction and complex formation is not observed in these cases, the affinity constant falling from $5 \times 10^{7} m^{-1}$ for hen lysozyme to an undetectable level for His 121 variants. The conformation of lysozyme in the complex shows no significant change from the native structure determined from various crystal forms. The structure of the Fab does not differ significantly in the framework regions from other known Fab structures, suggesting no large conformational changes are associated with antigen binding, although confirmation of this observation must await the structure determination of Fab D1.3 in the absence of antigen.

(1) Amit, A.G., Mariuzza, R.A., Phillips, S.E.V. and Poljak, R.J. (1986) Science 233, 747-753.

02.1-2 THE CRYSTAL STRUCTURE OF AN ANTI-ARSONATE ANTIBODY. By M.B. Lascombe, P.M. Alzari, P. Saludjian and P. Tougard. Département d'Immunologie, Institut Pasteur, 75724 Paris, France.

We report the structure determination of the Fab fragment of a monoclonal anti-p-azophenylarsonate (Ars) antibody (R19.9). This fragment does not possess the major idiotypic specificity present in the anti-Ars antibodies of A/J mice.

A 3.5 Å electron density map has been calculated by the multiple isomorphous replacement method. The interpretation of this map has been carried out by the molecular replacement method using the model of Fab New as a search object in direct space. The results of the 2.8 Å restrained least squares refinement of the structure will be discussed.

02.1-1 CRYSTAL STRUCTURE OF RICIN-OR. ONE OF TWO TOXINS ISOLATED FROM CASTOR BEANS. W. R. Chang[1], L. Chen[1], J. Rose[1], C. H. Wei[3], M. Sax[1,4], and B. C. Wang[1,2]. Departments of [1]Crystallography and [2]Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, [3]Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830, and [4]Biocrystallography Laboratory, VA Medical Center, Pittsburgh, PA 15240, USA.

Two toxic proteins have been isolated from the small bean variety of castor beans. One is ricin-D (Robertus, et al., University of Texas, Austin, Texas, USA), the other is ricin-OR. Although both proteins have comparable toxicity and anti-tumor activity, they differ distinctly in some of their properties. For example, ricin-D binds galactose strongly, but ricin-OR does not. Ricin-D has been crystallized in space group $P2_12_12_1$ and ricin-OR in space groups C2 and P2. These toxins are disulfide-bonded heterodimers, composed of A- and B-polypeptide chains. While the A-chain (MW ∿30,000) is responsible for the toxicity of the toxin, the B-chain (MW ∿35,000) is necessary for binding the toxins to the cell membrane. Following an initial binding via the B-chain, the A-chain is translocated in the cell cytoplasm, where it catalytically inactivates the 60S ribosomal subunit leading eventually to cell death. Recent interest in ricin has been in the preparation of immunotoxins targeted specifically for the destruction of certain tumor cells. An electron density map of the C2 form of ricin-OR has been calculated to 3 Å based on a single isomorphous heavy atom derivative using Wang's ISIR program. A model showing the relative location of the domains and initial interpretations of the polypeptide chain will be presented.

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02.1-3 CHARACTERIZATION OF FAB FRAGMENTS FROM MURINE MONOCLONAL ANTIBODIES WITH ACTIVITY TOWARD SINGLE-STRANDED DNA OR FLUORESCEIN. By A.B. Edmundson, J.N. Herron, X.-M. He, A.L.Gibson and E.W. Voss, Jr., Department of Biology, University of Utah, Salt Lake City, UT 84112 and Department of Microbiology, University of Illinois, Urbana, IL 61801.

Fab fragments from monoclonal antibodies reactive with single-stranded DNA (BV04-01) and fluorescein (4-4-20) crystallized in the same space group (triclinc P1) with almost identical unit cell dimensions (the mean R_F for 5-A data was about 34%). These observations were surprising because different solvents (ammonium sulfate and 2-methyl-2,4-pentanediol (MPD)) were used for crystallization. Moreover, the BV04-01 Fab was unliganded and the 4-4-20 Fab was co-crystallized with fluorescein. When co-crystallized with a trinucleotide of deoxythymidine, the BV04-01 Fab assumed a monoclinic space group ($P2_1$). With the McPC 603 Fab as a starting model, the structure of the unliganded BV04-01 Fab is currently being solved to 2.7-A resolution by molecular replacement and phase extension procedures. Rotation function studies indicated that the orientation of the liganded 4-4-20 Fab was similar to that of the BV04-01 Fab. An investigation of the crystal structure of the 4-4-20 Fab has been initiated with the BV04-01 Fab as the starting model. In solutions of MPD at concentrations used for crystallization, the affinity of the 4-4-20 antibody for fluorescein was found to decrease 300-fold relative to its value ($\sim 10^{10} M^{-1}$) in aqueous solutions. This work was supported by Grant CA 19616 from The National Cancer Institute (to A.B.E.) and Grant AI 20960 (to E.W.V.).