

the 20-2.8 Å resolution range. No attempt has been made to locate solvent molecules. The antibody-antigen interface extends over a large area ( $\sqrt{20 \times 30 \text{ Å}}$ ), 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 $\rightarrow$ His 121 in some avian lysozymes disturbs this interaction and complex formation is not observed in these cases, the affinity constant falling from  $5 \times 10^7 \text{ m}^{-1}$  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.

02.1-1 CRYSTAL STRUCTURE OF RICIN-OR. ONE OF TWO TOXINS ISOLATED FROM CASTOR BEANS. W. R. Chang<sup>1</sup>, L. Chen<sup>1</sup>, J. Rose<sup>1</sup>, C. H. Wei<sup>1</sup>, M. Sax<sup>1,2</sup>, and B. C. Wang<sup>1,2</sup>. Departments of <sup>1</sup>Crystallography and <sup>2</sup>Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, <sup>3</sup>Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830, and <sup>4</sup>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<sub>1</sub>2<sub>1</sub>2<sub>1</sub> 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  $\sim$ 30,000) is responsible for the toxicity of the toxin, the B-chain (MW  $\sim$ 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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02.1-2 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.

02.1-3 CHARACTERIZATION OF FAB FRAGMENTS FROM MURINE MONOCLONAL ANTIBODIES WITH ACTIVITY TOWARD SINGLE-STRANDED DNA OR FLUORESCIEIN. 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 (triclinic P1) with almost identical unit cell dimensions (the mean  $R_f$  for 5-Å 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<sub>1</sub>). With the McPC 603 Fab as a starting model, the structure of the unliganded BV04-01 Fab is currently being solved to 2.7-Å 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 ( $\sim 10^{10} \text{ M}^{-1}$ ) 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.).