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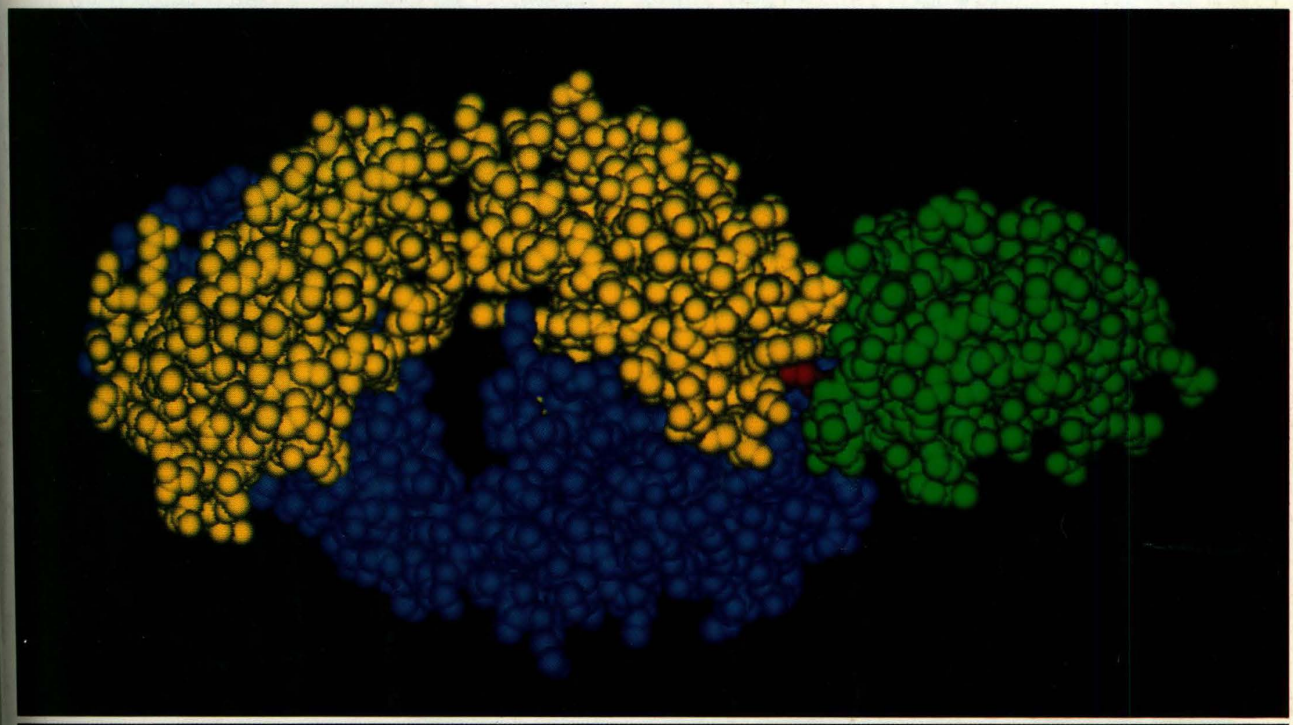

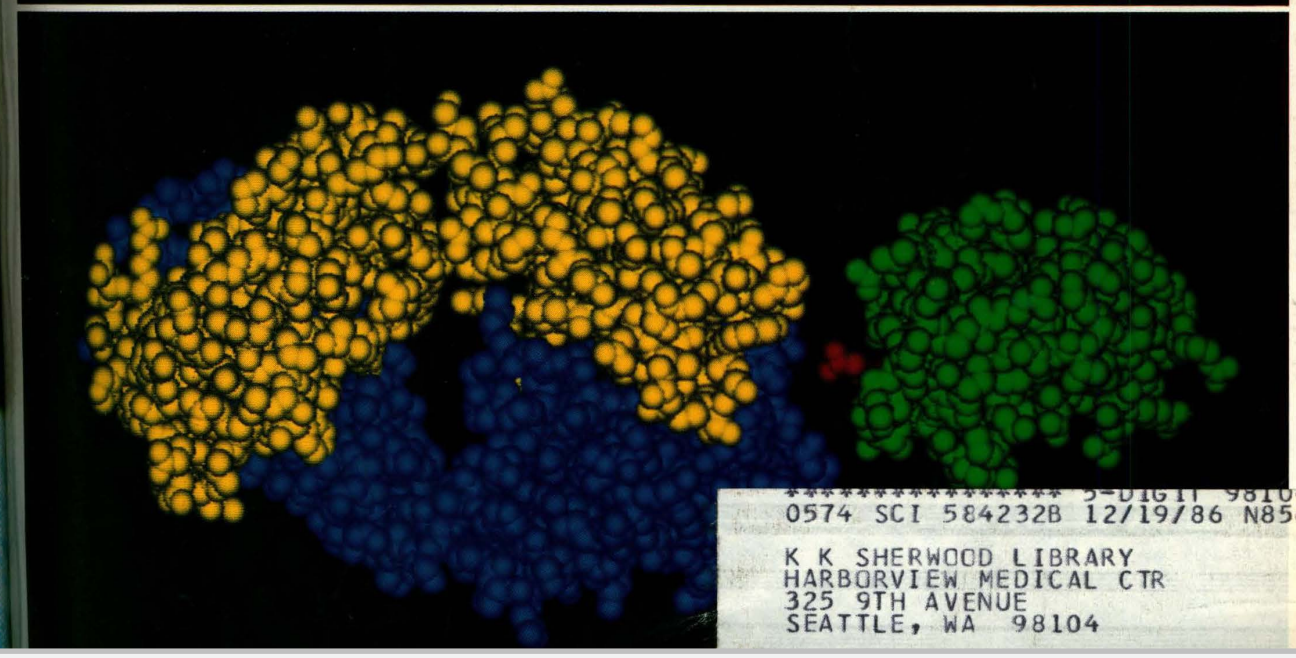

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■ The American Association for the Advancement of Science was founded in 1848 and incorporated in 1874. Its objects are to further the work of scientists, to facilitate cooperation among them, to foster scientific freedom and responsibility, to improve the effectiveness of science in the promotion of human welfare, and to increase public understanding and appreciation of the importance and promise of the methods of science in human progress.

COVER (Upper) Three-dimensional structure of an antigen-antibody complex. The antigen is lyzozyme (green, with a protruding residue, glutamine-121 in red). (Lower) The antigen and the antibody have been pulled apart to reveal their complementary contacting surfaces. See pages 747 and 755. [A. G. Amit *et al.*, Institut Pasteur, Paris, France]

Three-Dimensional Structure of an Antigen-Antibody Complex at 2.8 Å Resolution

A. G. Amit, R. A. Mariuzza, S. E. V. Phillips, R. J. Poljak

The 2.8 Å resolution three-dimensional structure of a complex between an antigen (lysozyme) and the Fab fragment from a monoclonal antibody against lysozyme has been determined and refined by x-ray crystallographic techniques. No conformational changes can be observed in the tertiary structure of lysozyme compared with that determined in native crystalline forms. The quaternary structure of Fab is that of an extended conformation. The antibody combining site is a rather flat surface with protuberances and depressions formed by its amino acid side chains. The antigen-antibody interface is tightly packed, with 16 lysozyme and 17 antibody residues making close contacts. The antigen contacting residues belong to two stretches of the lysozyme polypeptide chain: residues 18 to 27 and 116 to 129. All the complementarity-determining regions and two residues outside hypervariable positions of the antibody make contact with the antigen. Most of these contacts (10 residues out of 17) are made by the heavy chain, and in particular by its third complementarity-determining region. Antigen variability and antibody specificity and affinity are discussed on the basis of the determined structure.

THE BINDING OF FOREIGN ANTIGENS TO COMPLEMENTARY structures on the surface of B and T lymphocytes represents the initial step in the sequence of events leading to activation of the immune system. The receptor molecule on the surface of B lymphocytes responsible for antigen recognition is membrane immunoglobulin. A mature B cell produces and inserts into its plasma membrane only limited amounts of a single kind of immunoglobulin. Contact with antigen results in the expansion of B cell clones specific for that antigen and in their differentiation into plasma cells capable of producing and secreting large amounts of antibody of the same specificity (monoclonal antibody).

Antibody molecules of the immunoglobulin G (IgG) class, the most abundant in normal serum, are composed of two identical light (L) and two identical heavy (H) polypeptide chains. The amino terminal regions of the H and L chains, termed V_H and V_L, are each about 110 amino acids long and have variable (and homologous) amino acid sequences. The constant (C) half of the L chain, C_L, and the constant regions C_H1, C_H2, and C_H3 of the H chain, each about 100 amino acids long, have homologous sequences that belong to one of a few classes (κ and λ for L chains; μ, δ, γ, ϵ, and α for H chains). The V_H and V_L regions each contain three hypervariable or complementarity-determining regions (CDR1, CDR2, and CDR3) responsible for antigen recognition. These are flanked by less variable (FR1, FR2, FR3, and FR4) "framework" regions (*1*).

Present understanding of the three-dimensional structure of antibody combining sites is based on x-ray diffraction studies of myeloma immunoglobulins as reviewed (*2*). These have shown that the conformation of combining sites is determined by the amino acid sequences, unique to each different antibody, of the CDR's. The structures of two complexes of antigen-binding fragments (Fab) of myeloma immunoglobulins with small ligands have also been determined (*3, 4*). Although these studies resulted in useful models for ligand-antibody interactions, they are insufficient to establish unequivocally the precise size and shape of antibody combining sites, the nature and extent of antigen-antibody interactions, and the occurrence of possible conformational changes (if any) in the antibody after antigen binding. In addition, the precise structure of antigenic determinants on protein molecules remains to be determined (*5*). Equally important are questions concerning the nature of possible conformational changes in the complexed antigen and the effect of single amino acid substitutions on antigenic specificity and antigen recognition by the antibody.

We have recently determined the three-dimensional structure of an antigen-antibody complex, one between lysozyme and the Fab fragment of a monoclonal antibody to hen egg white lysozyme, at 6 Å resolution (*6*). We have since extended the resolution of the x-ray structure determination to 2.8 Å, and now present a complete description of antigen-antibody interactions in the complex.

Structure determination. The production of hybrid cell lines secreting murine monoclonal antibody to hen egg white lysozyme, and the purification, crystallization (*7*), and 6 Å resolution crystal structure determination (*6*) of the complex between Fab D1.3 and lysozyme have been described. Crystals grown from solutions containing 15 to 20 percent polyethylene glycol 8000 at *p*H 6.0 are monoclinic, space group $P2_1$, with $a = 55.6$, $b = 143.4$, $c = 49.1$ Å, $\beta = 120.5°$, and one molecule of complex per asymmetric unit.

Three heavy atom isomorphous derivatives were prepared with $(NH_4)_2PtCl_4$, $K_3F_5UO_2$, and *p*-hydroxymercuribenzenesulfonate. X-ray intensities were measured to 2.8 Å resolution with the use of a four-circle automatic diffractometer. Heavy atom sites were refined in alternate cycles of phasing and refinement (*8*); isomorphous phases, including anomalous scattering contributions (*9*), were calculated. The mean figure of merit (*10*) to 2.8 Å resolution was 0.47 for 15592 reflections. The electron density map calculated from these data was not readily interpretable, presumably because of lack of isomorphism of the heavy atom derivatives affecting phase determination at high resolution. The phases were further refined by a density modification technique (*11*) with a molecular envelope traced from the Fab-lysozyme model determined at 6 Å resolution (*6*). The resulting phases depend only on the observed data and the overall shape and position of the complex, but are independent of the detailed conformation of the previous model (*6*). The resulting

A. G. Amit, R. A. Mariuzza, and R. J. Poljak are in the Département d'Immunologie, Institut Pasteur, 75724 Paris Cedex 15, France. S. E. V. Phillips is in the Astbury Department of Biophysics, University of Leeds, Leeds, United Kingdom.

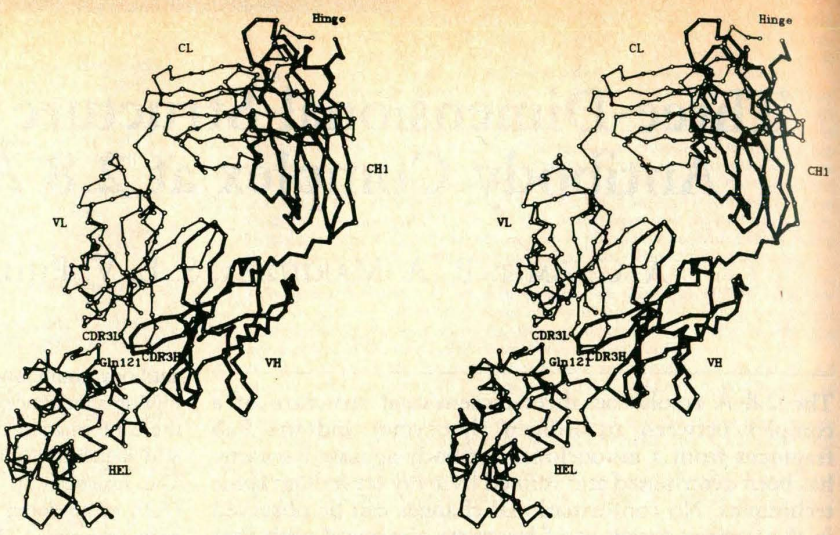

Fig. 1. Stereo diagram of the Cα skeleton of the complex. Fab is shown (upper right) with the heavy and light chains with thick and thin bonds, respectively. The lysozyme active site is the cleft containing the label HEL. Antibody-antigen interactions are most numerous between lysozyme and the heavy chain CDR loops.

electron density map was much improved, and an atomic model was fitted to it on an Evans and Sutherland PS300 interactive graphics system with the use of the program FRODO (*12*). The amino acid sequence of Fab D1.3 was derived from the corresponding light and heavy chain complementary DNA (cDNA) sequences (*13*). Of the 562 amino acid residues in the complex, 24 of those in the constant regions could not be located in the initial map. The atomic coordinates were submitted to alternate cycles of restrained crystallographic least-squares refinement (*14*) and model building. The model was checked in the later stages of refinement by sequentially omitting segments of the polypeptide chain (up to 20 percent of the total) and rebuilding them in maps phased from the remainder of the structure in combination with isomorphous replacement data (*15*). All residues have now been located, and the current crystallographic R factor is 0.28 for all data in the 20 to 2.8 Å resolution range. ($R = \Sigma \mid |Fo| - |Fc| \mid / \Sigma |Fo|$, where Fo, Fc are the observed and calculated structure factors of x-ray reflections.) No attempt was made to locate solvent molecules. Two isotropic temperature factors were used for each residue, one for the main chain atoms, and another for the side chain atoms. Stereochemical restraints were adjusted to give a standard deviation in C–C bonds of ±0.03 Å. No restraints were applied between residues across the antibody-antigen interface. Atomic coordinates will be deposited at Brookhaven Protein Data Bank after higher resolution and crystallographic refinement.

Conformation of the complexed antigen and of the Fab. The overall structure of the complex at 2.8 Å resolution (Fig. 1) confirms the results of the 6 Å resolution study (*6*). The assignment of the H and L polypeptide chains of Fab is unchanged. The closely packed β sheets are seen in Fab as are the helical and β-sheet structures surrounding the active site in lysozyme. The Fab appears in an almost fully extended conformation, with a definite separation between the variable (V) and constant (C) domains. With the exception of this difference in quaternary structure, Fab D1.3 compares closely to other known Fab's (*4, 16*), except in the CDR loops. Predicted structures for D1.3 (*17*) based on other Fab's also agree well with the determined structure in the framework β-sheet regions and in some, but not all, of the CDR loops. The relative disposition of the variable subunits of the H chain (V_H) and of the L chain (V_L), is unaltered, indicating no change in quaternary structure in the V domain resulting from antigen binding. Since the crystal structure of the unliganded Fab D1.3 has not been determined, detailed changes in antibody conformation remain to be verified. However, the similarity with other Fab structures suggests that possible conformational changes would be small. This observation is in agreement with that made by nuclear magnetic resonance (NMR) on the unliganded and hapten-liganded (dinitrophenol) mouse myeloma protein MOPC315 (*18*).

A least-squares fit of Cα atoms of lysozyme in the complex and native lysozyme refined at 1.6 Å in its tetragonal crystal form (*19*) gives a root-mean-square (rms) deviation of 0.64 Å between the two (see Fig. 2). Since the error in atomic positions in the complex can be estimated (*20*) to be approximately 0.6 Å, the difference is not significant. Furthermore, the largest changes (up to 1.6 Å) occur in regions remote from antibody contacts. Similar comparisons of native tetragonal lysozyme with other crystal forms gave rms deviations of 0.88 Å with triclinic lysozyme refined from x-ray and neutron diffraction data (*21*) and 0.46 Å for orthorhombic lysozyme determined at physiological temperature (*22*). Some differences in side chain conformation are observed between tetragonal and complexed lysozyme, but close examination with computer graphics revealed these to be similar to differences observed between different crystal structures of native lysozyme. Thus, complex formation with antibody D1.3 produces no more distortion of the structure of lysozyme than does crystallization.

The antigen-antibody interface. The interface between antigen and antibody extends over a large area with maximum dimensions of about 30 by 20 Å (Figs. 3 and 4). The antibody combining site appears as an irregular, rather flat surface with protuberances and depressions formed by the amino acid side chains of the CDR's of V_H and V_L. In addition, there is a small cleft between the third CDR's of V_H and V_L, corresponding to the binding site characterized in hapten-antibody complexes (*3, 4*). The cleft accepts the side chain Gln 121 of lysozyme although this is not the center of the antigen-antibody interface (Fig. 3).

The lysozyme antigenic determinants recognized by D1.3 are made up of two stretches of polypeptide chain, comprising residues 18 to 27 and 116 to 129, distant in the amino acid sequence but adjacent on the protein surface. All six CDR's interact with the antigen and in all, 16 antigen residues make close contacts with 17 antibody residues (Tables 1 and 2). Two antibody contacting residues, V_L Tyr 49 and V_H Thr 30, are just outside segments commonly defined as CDR's [sequence numbers are as in Kabat *et al.* (*1*) except for V_H CDR3; see Tables 2 and 3]. V_H Thr 30 is a constant or nearly constant residue in mouse H chain subgroups I and II, as is V_L Tyr 49 in mouse kappa chains. While the interaction of V_L Tyr 49 with antigen is relatively weak (one van der Waals

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