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THE STRUCTURAL BASIS OF ANTIGEN-ANTIBODY RECOGNITION

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PERSPECTIVES AND OVERVIEW

Antibody molecules, acting as specific antigen receptors at lymphocyte surfaces or in solution in serum and other biological fluids, are a central

part of the immune defense systems of higher organisms. The immune system can normally discriminate between self and nonself antigens and between closely related structural forms of chemical molecules with a high specificity, which is based on the complementarity of close molecular interactions. Biochemical, immunochemical, and physicochemical studies of immunoglobulins and antibodies (reviewed in references 4, 7, 16, 28) have shown that the structural bases of antigen recognition reside in the Fab part of the antibody molecule. The complementarity-determining regions (CDRs) of the heavy (H) and light (L) polypeptide chains of immunoglobulins determine, by their hypervariable sequences, the antigen-binding specificity of antibody molecules.

X-ray crystallographic studies of ligand-antibody (Fab) complexes (5, 54; reviewed in 4, 16) provided the most detailed structural pictures of specific binding reactions at the combining sites of antibody molecules. The crystalline material for these X-ray diffraction studies was obtained from human and murine myeloma immunoglobulins. However, these studies were not sufficient to allow characterization of the combining site of antibodies because the ligands, vitamin K_1OH (5) and phosphorylcholine (54), are relatively small and do not contact all the combining site residues. Thus, for example, the fact that no conformational changes were observed in the complexed Fabs (5, 54) could be attributed to the insufficient number of contacts made by the ligands with the combining site, or to the fact that the antibodies were not highly specific or did not have a sufficiently high affinity constant ($K_A \sim 1 \times 10^5$ mol^{-1}) for those ligands. Equally important questions remained about the nature of antigenic determinants (or epitopes) recognized by antibody molecules. For example, are the epitopes recognized by antibodies made of continuous residues of the sequence of the antigen, or are they discontinuous, topographical features assembled by the three-dimensional folding of the antigen molecule? In addition, are there conformational changes in the antigen following complex formation with the antibody? How can antigen variations influence the specificity of antibodies? These and other questions could only be answered by the study of an antigen-antibody complex.

The advent of cellular hybridization techniques for producing cell lines secreting antibodies of predefined specificity (33) provided an experimental approach to these questions. We chose lysozyme as an antigen model because its three-dimensional structure is known to high resolution (10), and because it has been used as a model antigen in different laboratories (reviewed in 7). We expected a crystalline complex between the antigen, hen egg-white lysozyme (HEL), and the Fab fragment of a specific monoclonal anti-HEL antibody (acting as a monovalent antibody) to provide a structural model for the interpretation of an antigen-antibody interaction.

After a systematic exploration of 27 monoclonal anti-HEL antibodies we obtained such a complex in crystalline form and studied it by X-ray diffraction techniques as reviewed below.

We do not endeavor to present here an exhaustive review of antigen-antibody interactions or of the pertinent literature. Instead we discuss some of the conclusions of our recent crystal-structure determination of this antigen-antibody complex. We emphasize that all the experimental data that have been obtained by immunochemical studies with HEL and the specific anti-HEL monoclonal antibody D1.3 in solution (20) are in agreement with the observations and conclusions of the crystal structure analysis (2, 3), and thus confirm the relevance of this analysis in the interpretation of immunochemical phenomena.

THE THREE-DIMENSIONAL STRUCTURE OF ANTIGEN AND ANTIBODY IN THE COMPLEX

The production of hybrid cell lines secreting murine monoclonal anti-HEL antibodies, and the purification and crystallization of the complex between Fab D1.3 and lysozyme have been described (41). Crystals were grown from 15–20% polyethylene glycol 8000 solutions at pH 6.0. The three-dimensional crystal structure was solved to a resolution of 6 Å and subsequently to 2.8 Å. The conventional R value after high-resolution refinement is currently 0.28.

Overall Conformation of the Fab-Lysozyme Complex

A low-resolution (6 Å) electron-density map of the Fab-lysozyme complex revealed the typical domain structure of a Fab fragment (45) plus an additional globular region, corresponding to lysozyme, in close contact with the Fab. The α-carbon backbone of the previously determined hen-lysozyme structure could be fitted to the map using only rigid-body rotations and translations. The α-carbon backbone of the variable domain ($V_L + V_H$) of Fab New[1] (46, 47) was similarly fitted, but the constant domain ($C_H1 + C_L$) could be fitted only by rotation about an axis through residues 103L and 117H at the flexible switch regions. This changed the angle between the variable and constant domains from 137° in Fab New to about 180°, closer to the 166° angle observed in Fab Kol (23). The low-resolution model (resembling that shown in Figure 1, obtained at 2.8-Å resolution) showed that the interactions between Fab and lysozyme extend over a large area of about 20 × 30 Å.

The electron-density map of the complex at 2.8-Å resolution confirmed

[1] Human Fabs and myeloma immunoglobulins are labeled with the first three letters of the patient's surname.

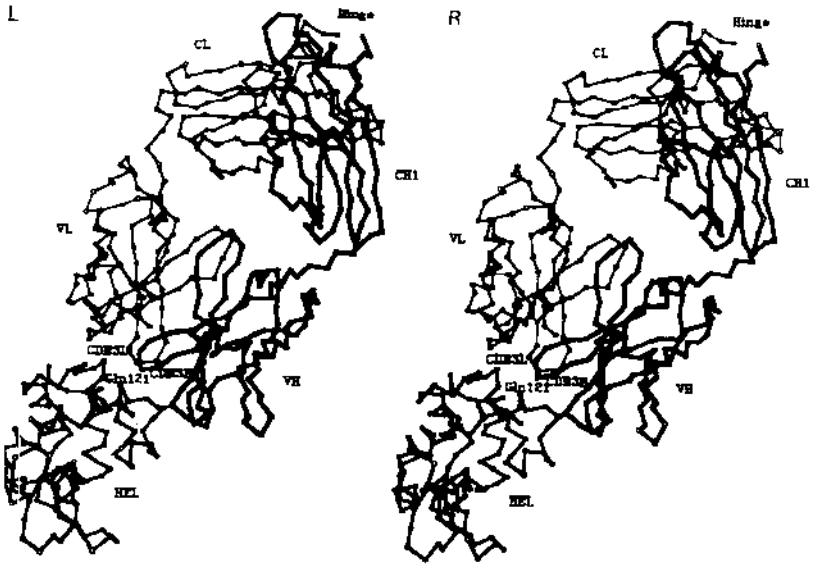

Figure 1 Stereo diagram of the α-carbon skeleton of the Fab-lysozyme complex. Fab is shown in *upper right*; the heavy and light chains are shown with *thick* and *thin* bonds, respectively. The lysozyme active site is the cleft containing the label HEL. Antibody-antigen interactions are most numerous between the lysozyme and the heavy chain CDR loops. (Reproduced from Reference 3, with permission.)

the results of the 6-Å resolution study, and also clearly showed the detailed conformation of the amino-acid side chains, allowing a complete description of the structure.

The Antigen-Antibody Interface and the Nature of the Antibody Combining Site

The site recognized by D1.3 is made up of two stretches of the polypeptide chain of lysozyme, residues 18–27 and 116–129, which are distant in the amino-acid sequence but adjacent on the protein surface (Table 1).

The antibody combining site appears as an irregular, relatively flat surface with protuberances and depressions formed by the amino-acid side chains of the CDRs of V_H and V_L. In addition, there is a small cleft between the third CDRs of V_H and V_L, corresponding to the binding site characterized in hapten-antibody complexes (5, 54). Although the cleft is not the geometrical center of the antigen-antibody interface, it binds the side chain of Gln121 of lysozyme (see Figure 2). All six CDRs interact with the antigen; 16 antigen residues make close contacts with 17 antibody residues (Table 2). Hydrogen exchange experiments with polyclonal anti-

Table 1 Lysozyme residues in contact with antibody[a]

Lysozyme residues	Number of antibody residues in contact
Asp18	1 L chain
Asn19	2 H, L
Arg21	1 H
Gly22	4 H(3), L
Tyr23	2 H
Ser24	1 H
Leu25	1 L
Asn27	1 H
Lys116	3 H
Gly117	6 H
Thr118	2 H
Asp119	2 H
Val120	1 H
Gln121	5 H(1), L(4)
Ile124	2 L
Leu129	1 L

[a] From Reference 3.

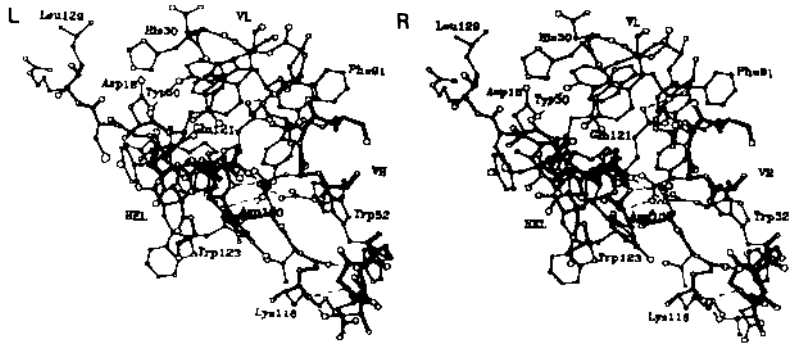

Figure 2 Stereo diagram of the antibody-antigen interface in an orientation similar to that in Figure 1. All atoms are shown for residues involved in the interaction. Heavy and light main chains are indicated by *thick* and *thin* bonds, and hydrogen bonds are indicated by *dotted lines*. Most lysozyme residues lie below the diagonal from *top left* to *lower right* of the diagram. (Reproduced from Reference 3, with permission.)

$(Glu,Ala,Tyr)_n$ sheep and rabbit antibodies (39) indicated 16–19 apparently site-associated amide hydrogens that did not exchange out from liganded Fab, and an additional 6–7 amide hydrogens whose exchange was retarded upon ligand binding. These numbers are in agreement with the number of antibody-contacting residues in the lysozyme-Fab complex described here

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