Perspective

Structural Basis for Antigen-Antibody Recognition

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WHEN THE FIRST CRYSTAL AND MOLECULAR STRUCTURES of immunoglobulins and their fragments were determined more than 12 years ago, there was much excitement. Not only were long-standing, well-defined questions answered but new routes of thinking about antibody function opened (*1–7*). These crystal studies showed that the polypetide chains (heavy and light chains) of immunoglobulin molecules are folded into globular domains, four (or five) in the heavy (H) and two in the light (L) chain, which are connected by extended peptide segments like pearls on a string. All domains exhibit similar folding characterized by two β sheets of polypeptide chains.

The immunoglobulin fold turned out to be a structural pattern found also in some functionally unrelated proteins like superoxide dismutase (*8*) or α-amylase inhibitor (*9*). Nature's repertoire of stable and fast-folding polypeptide motifs seems to be limited. The repertoire of structures of integral membrane proteins is likely to be even more restricted to assemblies of transmembrane helices (*10*) or to membrane spanning β strands arranged in barrels (*11*). However, there are as many variants as there are amino acid sequence variations permitted by the structural pattern. Some structural patterns seem to exhibit enormous plasticity to accommodate sequence variations while preserving the essential secondary structural elements and their interactions. As the surface determines binding and association properties toward small and large ligands, drastic functional differences between members in the same protein family are generated. Another means of modulating existing or creating new functions is by covalent joining or nonbonded association of structural domains, which may have the same or different folding patterns and different functional properties.

Immunoglobulins are good examples of both phenomena. One aspect of antibody function is the seemingly unlimited repertoire of antigen binding specificities. Hence it is surprising that antibody-antigen interaction is restricted to a small part of the antibody, an area at the tips of the Y-shaped molecule, to which the variable domains of both heavy and light chains contribute. This had been suggested by early amino acid sequence work (*12*), which revealed hypervariability of noncontiguous polypeptide segments and subsequently were found to be spatially adjacent at the tips of the Fab fragments of the antibody molecule. In addition, haptens were bound in crevices formed by parts of these hypervariable segments. The structural work showed that hapten binding seemed to follow the rules of structural complementarity, occurring with minimal structural adaptation; it then became obvious that single residue exchanges in the contact area could alter hapten binding properties profoundly.

Analysis of the structure of antibody genes had revealed the genetic basis of the hypervariability of protein segments involved in antigen binding (*13*). Some of these segments are encoded by separate genetic elements that somatically recombine with other elements so that the repertoire of antibody specificities is increased even further.

However, small haptenic ligands differ in many respects from macromolecular antigens, particularly in the ability to activate effector systems. Had previous structural studies missed an essential feature that might be revealed by studying antigen-antibody complexes? The advent of hybridoma technology (*14*) opened the way to an answer; but the path from a hybridoma cell line to a three-dimensional structure of an antibody-antigen complex is long and entails protein preparation, purification and characterization by sequence, crystallization, and crystallographic analysis.

Amit *et al.* describe in this issue (page 747) their successful experiments culminating in the first description at the atomic level of an antigen-antibody, or better an antigen-Fab fragment, complex. This group, headed by Roberto Poljak, chose lysozyme as an antigen whose covalent and spatial structure is well known. Many lysozyme variants are available and the antigenic structure of this enzyme has been characterized (*15*). Instead of using the intact antibody, which is very resistant to crystallization presumably because of the flexible connection between Fab arms and the Fc stem part (*6*), Poljak's group prepared the Fab fragment from a monoclonal antibody, which they call Fab D1.3.

Their study of the molecular structure of this antibody-lysozyme complex shows that, unlike the haptens, there is an extensive contact area between antigen and antigen-combining region of the antibody. Thus, 17 amino acid residues of the antibody and 16 of lysozyme interact tightly by van der Waals forces and hydrogen bonds. The surfaces of antigen and antibody are complementary and pack with a density similar to that found in the interior of protein molecules. Exchange of some of these residues would sterically interfere with packing or bonding and therefore cannot be tolerated. This explains qualitatively the observed binding pattern of different avian lysozymes to the Fab D1.3. The general nature of protein-protein interactions in this antibody-antigen complex resembles what has been seen in other systems, such as the interaction between proteases and their cognate natural inhibitors (*16*). Conformational changes that may occur upon complex formation are small and probably below the limits of detectability of the present analyses. Large conformational changes would indeed be opposed by the stabilization energy of the protein and are likely to occur upon relatively weak antigen-antibody interaction.

The central question about function and structural changes of Fab must remain unanswered because a crystal structure of the free antibody is not yet available. However, a detailed view of the interacting residues in the lysozyme-Fab complex shows that the lysozyme residues belong to two surface loops (16 residues altogether), which are spatially adjacent but not contiguous. Lysozyme has been intensively studied and it has been proposed that only three antigenic sites exist in lysozyme (*17*) but these do not agree with the observed epitopes in the Fab D1.3–lysozyme complex. In contrast, the view that a protein is multideterminant, and that any residue accessible from the surface may be involved in antigen-antibody recognition appears plausible (*15*). The observation of two noncontiguous polypeptide segments as antigenic determinants also illustrates the limits of the use of peptides to map the antigenic structure of proteins.

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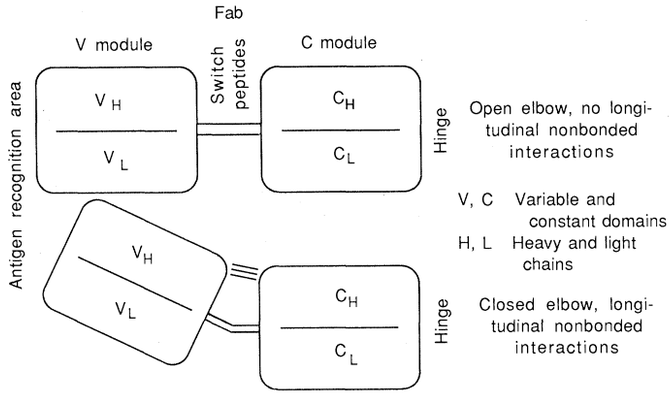
A somewhat similar problem also exists with the recently proposed correlation between thermal mobility and antigenicity (*18*) in that the observed contacting segments show only moderate mobility in the crystal structure of free lysozyme (*19*). In principle, such a correlation appears plausible as a product of the obvious correlation between antigenicity and surface exposure (*20*) and the well-established correlation of surface exposure with disorder and mobility. However, the experimental verification continues to rest principally on the mapping of the antigenic determinants with the antibodies to peptides.

In the Fab D1.3 all hypervariable segments (three from the light and three from the heavy chain) contribute to antigen binding, with a particularly large number of contacts provided by residues of the third hypervariable segment of the heavy chain. This heavy chain region has high variability also in length due to somatic recombination processes and imprecise joining at the D_H–J_H junction. The very high proportion of aromatic residues (9 out of 17) in the binding region is noteworthy. We may add two arginines whose guanidinium groups also have delocalized electron systems. The polarizability of these residues may allow stronger binding and could explain the abundance of aromatic residues in the hypervariable segments of antibodies (*21*).

In view of the tight complementary fit almost any exchange of the 17 contacting residues of the Fab will affect binding specificity. Within the framework of this structure we may expect as many as 20^{17} antibodies with differing specificities, enough to fill the universe. This simplistic view only illustrates the enormous potential of antibodies to exhibit different surface topologies. The question of the structural basis of antibody specificity and diversity therefore seems to be reducible to a problem of the tolerance of the basic structural immunoglobulin fold toward residue exchanges in the hypervariable segments, as mentioned earlier. Indeed all antibodies and Fab fragments, even from different species whose spatial structures have been determined, show a closely similar framework despite large differences in the hypervariable segments. The immunoglobulin fold appears to be highly tolerant toward mutations in the combining loops. It should be remembered that a stable protein structure requires both inherent structural stability and sufficiently fast folding kinetics. The immunoglobulin fold must be very stable, considering also the latter aspect. A revealing experiment demonstrated that complementarity-determining regions can be transplanted between antibodies and carry with them the binding specificity of the donor antibody molecule (*22*).

The problems of conformational changes in the antibody upon antigen binding continues to be of great interest. Although a direct and detailed structural comparison of free and antigen-bound Fab is not yet available, the framework structure and the lateral domain association (V_H with V_L) of the Fab D1.3 in its complex with lysozyme appears to be as in other known immunoglobulins. However, the Fab has an open elbow angle and lacks nonbonded longitudinal interactions between the V_H–V_L and C_H–C_L modules, as shown in the figure.

The potential of antibodies to adopt a conformation with an open elbow was discovered more than 10 years ago when we analyzed the structures of the intact myeloma protein KOL and later its Fab fragment (*5, 6*). Other Fab fragments (*1–3*) had shown closed elbows. This observation led us to suggest that elbow bending (that is, opening and closing of the longitudinal nonbonded contacts between V_H and C_H) is part of a signal (if such a signal exists) to communicate antigen binding at one end of the Fab to the distal carboxyl terminus from where it might be propagated to the Fc stem part of the molecule (*6*). We suggested very specifically that a closed elbow angle may be characteristic for antigen-bound antibodies. This specific suggestion has now been falsified by the structural



work of Amit *et al.*, but not the underlying basic idea of elbow bending as a signal transfer mechanism. Similar to the antilysozyme Fab in the complex with lysozyme, the KOL immunoglobulin G and Fab molecules are characterized by an open elbow conformation and show a remarkable tight complementary packing of their antigen-binding loops against the hinge region of neighboring molecules in the crystal. As we suggested in later work (*5*), this packing may be a model of antibody-antigen interaction and the open elbow may be characteristic for antigen-bound antibodies.

More information about the basic and important problem of signal generation by antigen binding and signal transduction through the Fab part of the antibody molecule will soon be provided by crystallographic analysis of free and antigen-bound Fab (*23, 24*). Indeed a number of experiments suggest that such longitudinal signal transduction occurs in Fab (*3, 25*). This question is intimately bound up with the more general problem of the linkage of antigen binding and effector functions which are associated with the Fc parts (*25*). Present-day tools, specific antibodies and x-ray crystallographic techniques, may solve this kind of problem.

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