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Immunobiology: The Immune System in Health and Disease. 5th edition.

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The interaction of the antibody molecule with specific antigen

We have described the structure of the [antibody](#) molecule and how the V regions of the heavy and light chains fold and pair to form the [antigen-binding site](#). In this part of the chapter we will look at the antigen-binding site in more detail. We will discuss the different ways in which antigens can bind to antibody and address the question of how variation in the sequences of the antibody V domains determines the [specificity](#) for antigen.

3-6. Localized regions of hypervariable sequence form the antigenbinding site

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The V regions of any given [antibody](#) molecule differ from those of every other. Sequence [variability](#) is not, however, distributed evenly throughout the V regions but is concentrated in certain segments of the V region. The distribution of variable amino acids can be seen clearly in what is termed a variability plot ([Fig. 3.6](#)), in which the amino acid sequences of many different antibody V regions are compared. Three segments of particular variability can be identified in both the V_H and V_L domains. They are designated [hypervariable regions](#) and are denoted HV1, HV2, and HV3. In the light chains these are roughly from residues 28 to 35, from 49 to 59, and from 92 to 103, respectively. The most variable part of the domain is in the HV3 region. The regions between the hypervariable regions, which comprise the rest of the [V domain](#), show less variability and are termed the [framework regions](#). There are four such regions in each V domain, designated FR1, FR2, FR3, and FR4.

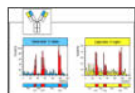


Figure 3.6

There are discrete regions of hypervariability in V domains. A variability plot derived from comparison of the amino acid sequences of several dozen heavy-chain and light-chain V domains. At each amino acid position the degree of variability is the ratio ([more...](#))

The [framework regions](#) form the β sheets that provide the structural framework of the domain, whereas the [hypervariable](#) sequences correspond to three loops at the outer edge of the β barrel, which are juxtaposed in the folded domain ([Fig. 3.7](#)). Thus, not only is sequence diversity concentrated in particular parts of the [V domain](#) but it is localized to a particular region on the surface of the molecule. When the V_H and V_L domains are paired in the [antibody](#) molecule, the hypervariable loops from each domain are brought together, creating a single hypervariable site at the tip of each arm of the molecule. This is the binding site for [antigen](#), the [antigen-binding site](#) or antibody combining site. The three hypervariable loops determine antigen [specificity](#) by forming a surface complementary to the antigen, and are more commonly termed the **complementarity-determining regions**, or CDRs (**CDR1**, **CDR2**, and **CDR3**). Because CDRs from both V_H and V_L domains contribute to the antigen-binding site, it is the combination of the heavy and the [light chain](#), and not either alone, that determines the final antigen specificity. Thus, one way in which the [immune system](#) is able to generate antibodies of different specificities is by generating different combinations of heavy- and light-chain V regions. This means of producing [variability](#) is known as [combinatorial diversity](#); we will encounter a second form of combinatorial diversity when we consider in Chapter 4 how the genes encoding the heavy- and light-chain V regions are created from smaller segments of DNA.

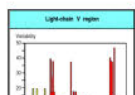


Figure 3.7

The hypervariable regions lie in discrete loops of the folded structure. When the hypervariable regions (CDRs) are

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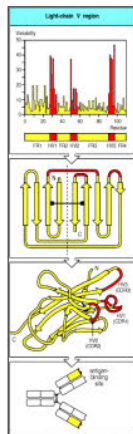


Figure 3.7

The hypervariable regions lie in discrete loops of the folded structure. When the hypervariable regions (CDRs) are positioned on the structure of a V domain it can be seen that they lie in loops that are brought together in the folded structure. In the [\(more...\)](#)

3-7. Antibodies bind antigens via contacts with amino acids in CDRs, but the details of binding depend upon the size and shape of the antigen

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In early investigations of [antigen](#) binding to antibodies, the only available sources of large quantities of a single type of [antibody](#) molecule were tumors of antibody-secreting cells. The antigen specificities of the tumor-derived antibodies were unknown, so many compounds had to be screened to identify ligands that could be used to study antigen binding. In general, the substances found to bind to these antibodies were haptens (see [Section 3-4](#)) such as phosphorylcholine or vitamin K₁. Structural analysis of complexes of antibodies with their hapten ligands provided the first direct evidence that the [hypervariable](#) regions form the [antigen-binding site](#), and demonstrated the structural basis of [specificity](#) for the hapten. Subsequently, with the discovery of methods of generating monoclonal antibodies (see [Appendix I, Section A-12](#)), it became possible to make large amounts of pure antibodies specific for many different antigens. This has provided a more general picture of how antibodies interact with their antigens, confirming and extending the view of antibody-antigen interactions derived from the study of haptens.

The surface of the [antibody](#) molecule formed by the juxtaposition of the CDRs of the heavy and light chains creates the site to which an [antigen](#) binds. Clearly, as the amino acid sequences of the CDRs are different in different antibodies, so are the shapes of the surfaces created by these CDRs. As a general principle, antibodies bind ligands whose surfaces are complementary to that of the antibody. A small antigen, such as a hapten or a short peptide, generally binds in a pocket or groove lying between the heavy- and light-chain V domains ([Fig. 3.8](#), left and center panels). Other antigens, such as a protein molecule, can be of the same size as, or larger than, the antibody molecule itself, and cannot fit into a groove or pocket. In these cases, the interface between the two molecules is often an extended surface that involves all of the CDRs and, in some cases, part of the framework region of the antibody ([Fig. 3.8](#), right panel). This surface need not be concave but can be flat, undulating, or even convex.

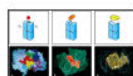


Figure 3.8

Antigens can bind in pockets or grooves, or on extended surfaces in the binding sites of antibodies. The panels in the top row show schematic representations of the different types of binding site in a Fab fragment of an antibody: left, pocket; center, [\(more...\)](#)

3-8. Antibodies bind to conformational shapes on the surfaces of antigens

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The biological function of antibodies is to bind to pathogens and their products, and to facilitate their removal from the body. An [antibody](#) generally recognizes only a small region on the surface of a large molecule such as a polysaccharide or protein. The structure recognized by an antibody is called an [antigenic determinant](#) or [epitope](#). Some of the most important pathogens have polysaccharide coats, and antibodies that recognize epitopes formed by the sugar subunits of these molecules are essential in providing immune protection from such pathogens. In many cases, however, the antigens that provoke an [immune response](#) are proteins. For example, protective antibodies against viruses recognize viral coat proteins. In such cases, the structures recognized by the antibody are located on

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3-9. Antigen-antibody interactions involve a variety of forces

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The interaction between an [antibody](#) and its [antigen](#) can be disrupted by high salt concentrations, extremes of pH, detergents, and sometimes by competition with high concentrations of the pure [epitope](#) itself. The binding is therefore a reversible noncovalent interaction. The forces, or bonds, involved in these noncovalent interactions are outlined in [Fig. 3.9](#).



Figure 3.9

The noncovalent forces that hold together the antigen:antibody complex. Partial charges found in electric dipoles are shown as δ^+ or δ^- . Electrostatic forces diminish as the inverse square of the distance separating the charges, whereas ([more...](#))

Electrostatic interactions occur between charged amino acid side chains, as in salt bridges. Interactions also occur between electric dipoles, as in hydrogen bonds, or can involve short-range van der Waals forces. High salt concentrations and extremes of pH disrupt [antigen-antibody](#) binding by weakening electrostatic interactions and/or hydrogen bonds. This principle is employed in the purification of antigens using affinity columns of immobilized antibodies, and vice versa for antibody purification (see [Appendix I, Section A-5](#)). Hydrophobic interactions occur when two hydrophobic surfaces come together to exclude water. The strength of a hydrophobic interaction is proportional to the surface area that is hidden from water. For some antigens, hydrophobic interactions probably account for most of the binding energy. In some cases, water molecules are trapped in pockets in the interface between antigen and antibody. These trapped water molecules may also contribute to binding, especially between polar amino acid residues.

The contribution of each of these forces to the overall interaction depends on the particular [antibody](#) and [antigen](#) involved. A striking difference between antibody interactions with protein antigens and most other natural protein-protein interactions is that antibodies possess many aromatic amino acids in their antigen-binding sites. These amino acids participate mainly in van der Waals and hydrophobic interactions, and sometimes in hydrogen bonds. In general, the hydrophobic and van der Waals forces operate over very short ranges and serve to pull together two surfaces that are complementary in shape: hills on one surface must fit into valleys on the other for good binding to occur. In contrast, electrostatic interactions between charged side chains, and hydrogen bonds bridging oxygen and/or nitrogen atoms, accommodate specific features or reactive groups while strengthening the interaction overall.

For example, in the complex of hen egg-white lysozyme with the [antibody](#) D1.3 ([Fig. 3.10](#)), strong hydrogen bonds are formed between the antibody and a particular glutamine in the lysozyme molecule that protrudes between the V_H and V_L domains. Lysozymes from partridge and turkey have another amino acid in place of the glutamine and do not bind to the antibody. In the high-affinity complex of hen egg-white lysozyme with another antibody, HyHel5 (see [Fig. 3.8c](#)), two salt bridges between two basic arginines on the surface of the lysozyme interact with two glutamic acids, one each from the V_H CDR1 and CDR2 loops. Again, lysozymes that lack one of the two arginine residues show a 1000-fold decrease in affinity. Although overall surface complementarity must play an important part in [antigen-](#)

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Figure 3.10
The complex of lysozyme with the antibody D1.3. The interaction of the Fab fragment of D1.3 with hen egg-white lysozyme is shown, with the lysozyme in blue, the heavy chain in purple and the light chain in yellow. A glutamine residue of lysozyme, shown [\(more...\)](#)

Summary

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X-ray crystallographic analysis of [antigen:antibody](#) complexes has demonstrated that the [hypervariable](#) loops (complementarity-determining regions) of immunoglobulin V regions determine the [specificity](#) of antibodies. With protein antigens, the antibody molecule contacts the antigen over a broad area of its surface that is complementary to the surface recognized on the antigen. Electrostatic interactions, hydrogen bonds, van der Waals forces, and hydrophobic interactions can all contribute to binding. Amino acid side chains in most or all of the hypervariable loops make contact with antigen and determine both the specificity and the affinity of the interaction. Other parts of the V region play little part in the direct contact with the antigen but provide a stable structural framework for the hypervariable loops and help determine their position and conformation. Antibodies raised against intact proteins usually bind to the surface of the protein and make contact with residues that are discontinuous in the primary structure of the molecule; they may, however, occasionally bind peptide fragments of the protein, and antibodies raised against peptides derived from a protein can sometimes be used to detect the native protein molecule. Peptides binding to antibodies usually bind in the cleft between the V regions of the heavy and light chains, where they make specific contact with some, but not necessarily all, of the hypervariable loops. This is also the usual mode of binding for carbohydrate antigens and small molecules such as haptens.

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