

immuno biology 5

THE IMMUNE SYSTEM IN HEALTH AND DISEASE

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Distributors:

Inside North America: Garland Publishing, 29 West 35th Street,
New York, NY 10001-2299.

Inside Japan: Nankodo Co. Ltd., 42-6, Hongo 3-Chrome, Bunkyo-ku,
Tokyo, 113-8410, Japan.

Outside North America and Japan: Churchill Livingstone, Robert Stevenson House,
1-3 Baxter's Place, Leith Walk, Edinburgh, EH1 3AF.

ISBN 0 8153 3642 X (paperback) Garland

ISBN 0 4430 7098 9 (paperback) Churchill Livingstone

ISBN 0 4430 7099 7 (paperback) International Student Edition

Library of Congress Cataloging-in-Publication Data

Immunobiology : the immune system in health and disease / Charles A. Janeway, Jr. ...
[et al.].-- 5th ed.

p. cm.

Includes bibliographical references and index.

ISBN 0-8153-3642-X (pbk.)

1. Immunology. 2. Immunity. I. Janeway, Charles. II. Title.

QR181 .J454 2001

616.07'9--dc21

2001016039

This book was produced using QuarkXpress 4.11 and Adobe Illustrator 9.0

Published by Garland Publishing, a member of the Taylor & Francis Group,
29 West 35th Street, New York, NY 10001-2299.

Printed in the United States of America.

15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

General Library System
University of Wisconsin - Madison
728 State Street
Madison, WI 53706-1494
U.S.A.



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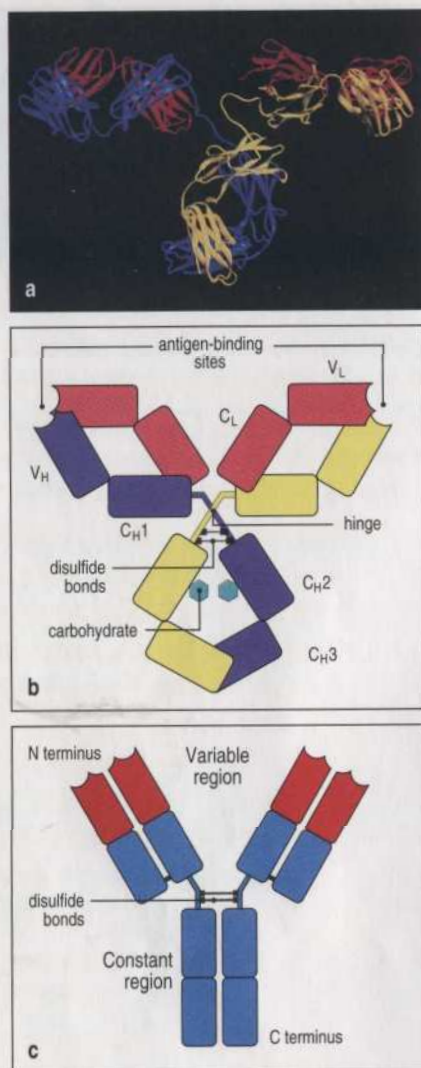


Fig. 3.1 Structure of an antibody molecule. Panel a illustrates a ribbon diagram based on the X-ray crystallographic structure of an IgG antibody, showing the course of the backbones of the polypeptide chains. Three globular regions form a Y. The two antigen-binding sites are at the tips of the arms, which are tethered to the trunk of the Y by a flexible hinge region. A schematic representation of the structure in a is given in panel b, illustrating the four-chain composition and the separate domains comprising each chain. Panel c shows a simplified schematic representation of an antibody molecule that will be used throughout this book. Photograph courtesy of A. McPherson and L. Harris.

The antigen-recognition molecules of T cells are made solely as membrane-bound proteins and only function to signal T cells for activation. These **T-cell receptors (TCRs)** are related to immunoglobulins both in their protein structure—having both V and C regions—and in the genetic mechanism that produces their great variability (see Section 1-10 and Chapter 4). However, the T-cell receptor differs from the B-cell receptor in an important way: it does not recognize and bind antigen directly, but instead recognizes short peptide fragments of pathogen protein antigens, which are bound to **MHC molecules** on the surfaces of other cells.

The MHC molecules are glycoproteins encoded in the large cluster of genes known as the **major histocompatibility complex (MHC)** (see Sections 1-16 and 1-17). Their most striking structural feature is a cleft running across their outermost surface, in which a variety of peptides can be bound. As we shall discuss further in Chapter 5, MHC molecules show great genetic variation in the population, and each individual carries up to 12 of the possible variants, which increases the range of pathogen-derived peptides that can be bound. T-cell receptors recognize features both of the peptide antigen and of the MHC molecule to which it is bound. This introduces an extra dimension to antigen recognition by T cells, known as **MHC restriction**, because any given T-cell receptor is specific not simply for a foreign peptide antigen, but for a unique combination of a peptide and a particular MHC molecule. The ability of T-cell receptors to recognize MHC molecules, and their selection during T-cell development for the ability to recognize the particular MHC molecules expressed by an individual, are topics we shall return to in Chapters 5 and 7.

In this chapter we focus on the structure and antigen-binding properties of immunoglobulins and T-cell receptors. Although B cells and T cells recognize foreign molecules in two distinct fashions, the receptor molecules they use for this task are very similar in structure. We will see how this basic structure can accommodate great variability in antigen specificity, and how it enables immunoglobulins and T-cell receptors to carry out their functions as the antigen-recognition molecules of the adaptive immune response.

The structure of a typical antibody molecule.

Antibodies are the secreted form of the B-cell receptor. An antibody is identical to the B-cell receptor of the cell that secretes it except for a small portion of the C-terminus of the heavy-chain constant region. In the case of the B-cell receptor the C-terminus is a hydrophobic membrane-anchoring sequence, and in the case of antibody it is a hydrophilic sequence that allows secretion. Since they are soluble, and secreted in large quantities, antibodies are easily obtainable and easily studied. For this reason, most of what we know about the B-cell receptor comes from the study of antibodies.

Antibody molecules are roughly Y-shaped molecules consisting of three equal-sized portions, loosely connected by a flexible tether. Three schematic representations of antibody structure, which has been determined by X-ray crystallography, are shown in Fig. 3.1. The aim of this part of the chapter is to explain how this structure is formed and how it allows antibody molecules to carry out their dual tasks—binding on the one hand to a wide variety of antigens, and on the other hand to a limited number of effector molecules and cells. As we will see, each of these tasks is carried out by separable parts of the molecule. The two arms of the Y end in regions that vary between different

3-2 Immunoglobulin heavy and light chains are composed of constant and variable regions.

The amino acid sequences of many immunoglobulin heavy and light chains have been determined and reveal two important features of antibody molecules. First, each chain consists of a series of similar, although not identical, sequences, each about 110 amino acids long. Each of these repeats corresponds to a discrete, compactly folded region of protein structure known as a protein domain. The light chain is made up of two such **immunoglobulin domains**, whereas the heavy chain of the IgG antibody contains four (see Fig. 3.1a). This suggests that the immunoglobulin chains have evolved by repeated duplication of an ancestral gene corresponding to a single domain.

The second important feature revealed by comparisons of amino acid sequences is that the amino-terminal sequences of both the heavy and light chains vary greatly between different antibodies. The variability in sequence is limited to approximately the first 110 amino acids, corresponding to the first domain, whereas the remaining domains are constant between immunoglobulin chains of the same isotype. The amino-terminal variable or **V domains** of the heavy and light chains (V_H and V_L , respectively) together make up the V region of the antibody and confer on it the ability to bind specific antigen, while the constant domains (**C domains**) of the heavy and light chains (C_H and C_L , respectively) make up the C region (see Fig. 3.1b, c). The multiple heavy-chain C domains are numbered from the amino-terminal end to the carboxy terminus, for example C_{H1} , C_{H2} , and so on.

3-3 The antibody molecule can readily be cleaved into functionally distinct fragments.

The protein domains described above associate to form larger globular domains. Thus, when fully folded and assembled, an antibody molecule comprises three equal-sized globular portions joined by a flexible stretch of polypeptide chain known as the **hinge region** (see Fig. 3.1b). Each arm of this Y-shaped structure is formed by the association of a light chain with the amino-terminal half of a heavy chain, whereas the trunk of the Y is formed by the pairing of the carboxy-terminal halves of the two heavy chains. The association of the heavy and light chains is such that the V_H and V_L domains are paired, as are the C_{H1} and C_L domains. The C_{H3} domains pair with each other but the C_{H2} domains do not interact; carbohydrate side chains attached to the C_{H2} domains lie between the two heavy chains. The two antigen-binding sites are formed by the paired V_H and V_L domains at the ends of the two arms of the Y (see Fig. 3.1b).

Proteolytic enzymes (proteases) that cleave polypeptide sequences have been used to dissect the structure of antibody molecules and to determine which parts of the molecule are responsible for its various functions. Limited digestion with the protease papain cleaves antibody molecules into three fragments (Fig. 3.3). Two fragments are identical and contain the antigen-binding activity. These are termed the **Fab fragments**, for Fragment antigen binding. The Fab fragments correspond to the two identical arms of the antibody molecule, which contain the complete light chains paired with the V_H and C_{H1} domains of the heavy chains. The other fragment contains no antigen-binding activity but was originally observed to crystallize readily, and for this reason was named the **Fc fragment**, for Fragment crystallizable. This fragment corresponds to the paired C_{H2} and C_{H3} domains and is the part of the antibody molecule that interacts with effector molecules and cells. The functional differences between heavy-chain isotypes lie mainly in the Fc fragment.

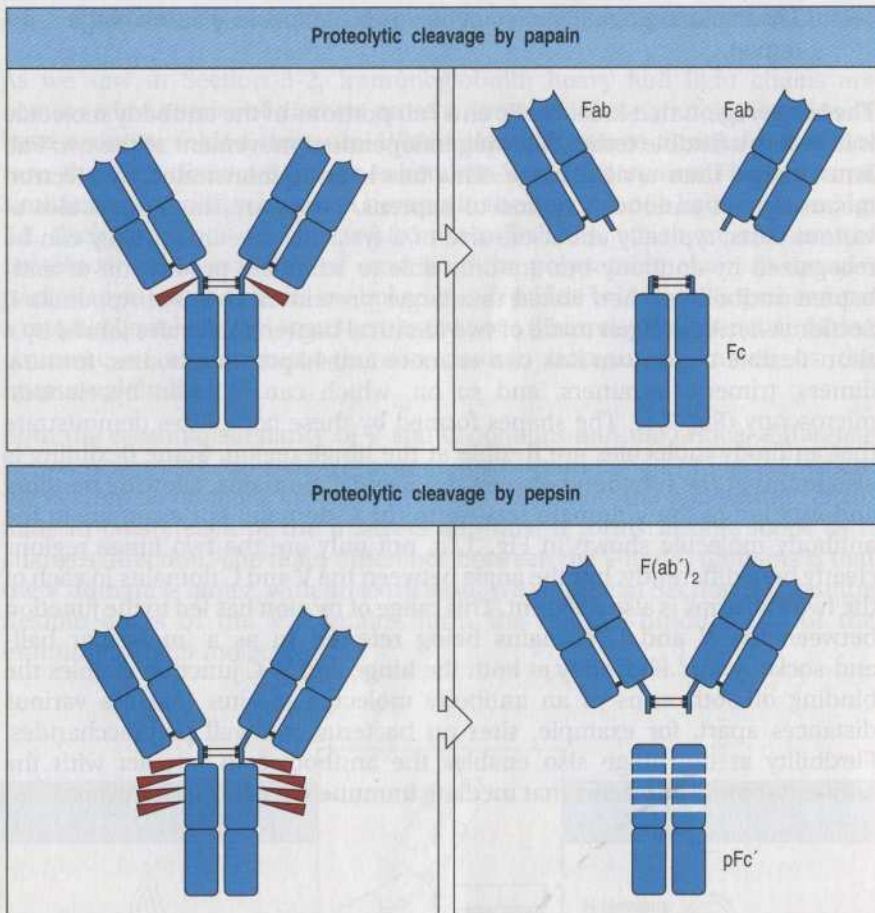


Fig. 3.3 The Y-shaped immunoglobulin molecule can be dissected by partial digestion with proteases.

Papain cleaves the immunoglobulin molecule into three pieces, two Fab fragments and one Fc fragment (upper panels). The Fab fragment contains the V regions and binds antigen. The Fc fragment is crystallizable and contains C regions. Pepsin cleaves immunoglobulin to yield one F(ab')₂ fragment and many small pieces of the Fc fragment, the largest of which is called the pFc' fragment (lower panels). F(ab')₂ is written with a prime because it contains a few more amino acids than Fab, including the cysteines that form the disulfide bonds.

The protein fragments obtained after proteolysis are determined by where the protease cuts the antibody molecule in relation to the disulfide bonds that link the two heavy chains. These lie in the hinge region between the C_H1 and C_H2 domains and, as illustrated in Fig. 3.3, papain cleaves the antibody molecule on the amino-terminal side of the disulfide bonds. This releases the two arms of the antibody as separate Fab fragments, whereas in the Fc fragment the carboxy-terminal halves of the heavy chains remain linked.

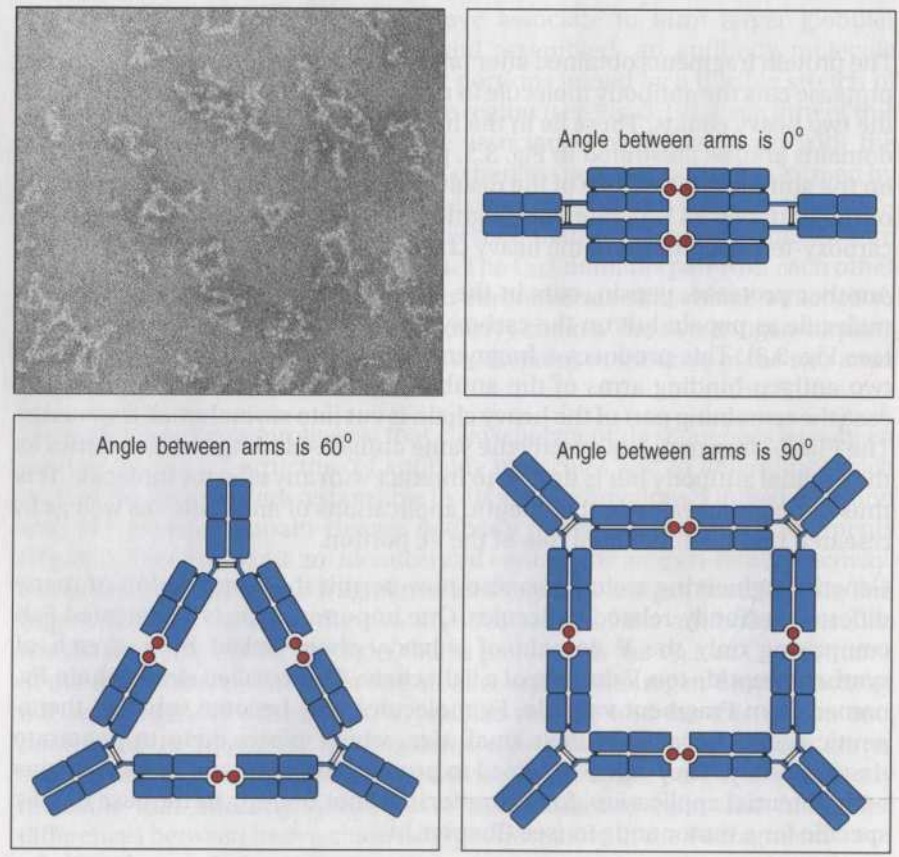
Another protease, pepsin, cuts in the same general region of the antibody molecule as papain but on the carboxy-terminal side of the disulfide bonds (see Fig. 3.3). This produces a fragment, the F(ab')₂ fragment, in which the two antigen-binding arms of the antibody molecule remain linked. In this case the remaining part of the heavy chain is cut into several small fragments. The F(ab')₂ fragment has exactly the same antigen-binding characteristics as the original antibody but is unable to interact with any effector molecule. It is thus of potential value in therapeutic applications of antibodies as well as in research into the functional role of the Fc portion.

Genetic engineering techniques also now permit the construction of many different antibody-related molecules. One important type is a truncated Fab comprising only the V domain of a heavy chain linked by a stretch of synthetic peptide to a V domain of a light chain. This is called **single-chain Fv**, named from **F**ragment **v**ariable. Fv molecules may become valuable therapeutic agents because of their small size, which allows them to penetrate tissues readily. They can be coupled to protein toxins to yield immunotoxins with potential application, for example, in tumor therapy in the case of a Fv specific for a tumor antigen (see Chapter 14).

3-4 The immunoglobulin molecule is flexible, especially at the hinge region.

The hinge region that links the Fc and Fab portions of the antibody molecule is in reality a flexible tether, allowing independent movement of the two Fab arms, rather than a rigid hinge. This has been demonstrated by electron microscopy of antibodies bound to **haptens**. These are small molecules of various sorts, typically about the size of a tyrosine side chain. They can be recognized by antibody but are only able to stimulate production of anti-hapten antibodies when linked to a larger protein **carrier** (see Appendix I, Section A-1). An antigen made of two identical hapten molecules joined by a short flexible region can link two or more anti-hapten antibodies, forming dimers, trimers, tetramers, and so on, which can be seen by electron microscopy (Fig. 3.4). The shapes formed by these complexes demonstrate that antibody molecules are flexible at the hinge region. Some flexibility is also found at the junction between the V and C domains, allowing bending and rotation of the V domain relative to the C domain. For example, in the antibody molecule shown in Fig. 3.1a, not only are the two hinge regions clearly bent differently, but the angle between the V and C domains in each of the two Fab arms is also different. This range of motion has led to the junction between the V and C domains being referred to as a 'molecular ball-and-socket joint.' Flexibility at both the hinge and V-C junction enables the binding of both arms of an antibody molecule to sites that are various distances apart, for example, sites on bacterial cell-wall polysaccharides. Flexibility at the hinge also enables the antibodies to interact with the antibody-binding proteins that mediate immune effector mechanisms.

Fig. 3.4 Antibody arms are joined by a flexible hinge. An antigen consisting of two hapten molecules (red balls in diagrams) that can cross-link two antigen-binding sites is used to create antigen:antibody complexes, which can be seen in the electron micrograph. Linear, triangular, and square forms are seen, with short projections or spikes. Limited pepsin digestion removes these spikes (not shown in the figure), which therefore correspond to the Fc portion of the antibody; the F(ab')₂ pieces remain cross-linked by antigen. The interpretation of the complexes is shown in the diagrams. The angle between the arms of the antibody molecules varies, from 0° in the antibody dimers, through 60° in the triangular forms, to 90° in the square forms, showing that the connections between the arms are flexible. Photograph (× 300,000) courtesy of N.M. Green.



3-5 The domains of an immunoglobulin molecule have similar structures.

As we saw in Section 3-2, immunoglobulin heavy and light chains are composed of a series of discrete protein domains. These protein domains all have a similar folded structure. Within this basic three-dimensional structure, there are distinct differences between V and C domains. The structural similarities and differences can be seen in the diagram of a light chain in Fig. 3.5. Each domain is constructed from two β sheets, which are elements of protein structure made up of strands of the polypeptide chain (β strands) packed together; the sheets are linked by a disulfide bridge and together form a roughly barrel-shaped structure, known as a β barrel. The distinctive folded structure of the immunoglobulin protein domain is known as the **immunoglobulin fold**.

Both the essential similarity of V and C domains and the critical difference between them are most clearly seen in the bottom panels of Fig. 3.5, where the cylindrical domains are opened out to reveal how the polypeptide chain folds to create each of the β sheets and how it forms flexible loops as it changes direction. The main difference between the V and C domains is that the V domain is larger, with an extra loop. We will see in Section 3-6 that the flexible loops of the V domains form the antigen-binding site of the immunoglobulin molecule.

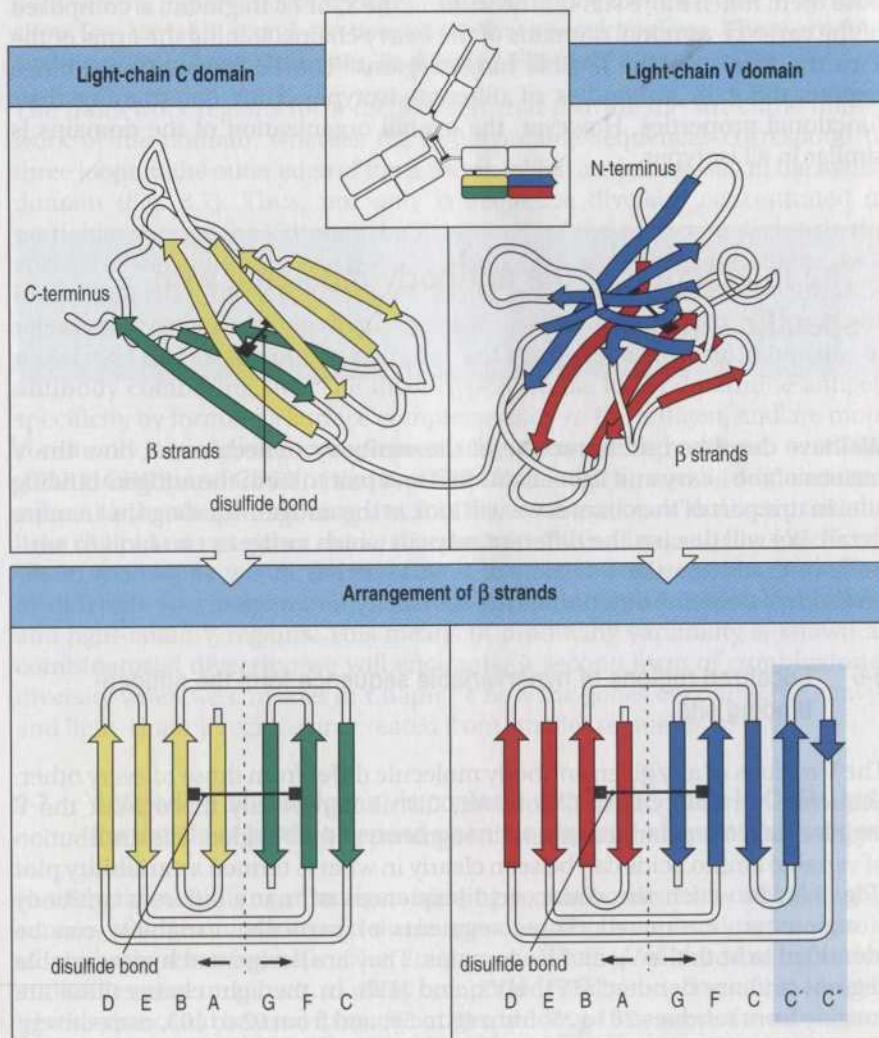


Fig. 3.5 The structure of immunoglobulin constant and variable domains. The upper panels show schematically the folding pattern of the constant (C) and variable (V) domains of an immunoglobulin light chain. Each domain is a barrel-shaped structure in which strands of polypeptide chain (β strands) running in opposite directions (antiparallel) pack together to form two β sheets (shown in yellow and green in the diagram of the C domain), which are held together by a disulfide bond. The way the polypeptide chain folds to give the final structure can be seen more clearly when the sheets are opened out, as shown in the lower panels. The β strands are lettered sequentially with respect to the order of their occurrence in the amino acid sequence of the domains; the order in each β sheet is characteristic of immunoglobulin domains. The β strands C' and C'' that are found in the V domains but not in the C domains are indicated by a blue shaded background. The characteristic four-strand plus three-strand (C-region type domain) or four-strand plus five-strand (V-region type domain) arrangements are typical immunoglobulin superfamily domain building blocks, found in a whole range of other proteins as well as antibodies and T-cell receptors.

Many of the amino acids that are common to C and V domains of immunoglobulin chains lie in the core of the immunoglobulin fold and are critical to its stability. For that reason, other proteins having sequences similar to those of immunoglobulins are believed to form domains of similar structure, and in many cases this has been demonstrated by crystallography. These **immunoglobulin-like domains** are present in many other proteins of the immune system, and in proteins involved in cell-cell recognition in the nervous system and other tissues. Together with the immunoglobulins and the T-cell receptors, they make up the extensive **immunoglobulin superfamily**.

Summary.

The IgG antibody molecule is made up of four polypeptide chains, comprising two identical light chains and two identical heavy chains, and can be thought of as forming a flexible Y-shaped structure. Each of the four chains has a variable (V) region at its amino terminus, which contributes to the antigen-binding site, and a constant (C) region, which determines the isotype. The isotype of the heavy chain determines the functional properties of the antibody. The light chains are bound to the heavy chains by many non-covalent interactions and by disulfide bonds, and the V regions of the heavy and light chains pair in each arm of the Y to generate two identical antigen-binding sites, which lie at the tips of the arms of the Y. The possession of two antigen-binding sites allows antibody molecules to cross-link antigens and to bind them much more stably. The trunk of the Y, or Fc fragment, is composed of the carboxy-terminal domains of the heavy chains. Joining the arms of the Y to the trunk are the flexible hinge regions. The Fc fragment and hinge regions differ in antibodies of different isotypes, thus determining their functional properties. However, the overall organization of the domains is similar in all isotypes.

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 antigen-binding site.

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.