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ANTIBODY-ANTIGEN COMPLEXES[1]

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INTRODUCTION

Antibodies are made in all vertebrates as part of the immune response to antigenic challenge by foreign substances. The diversity of this response is impressive: any foreign macromolecule can, under appropriate conditions, elicit an immune response. It has been estimated that humans can produce as many as 10 million different antibodies in the primary repertoire and that this may then be further expanded by several orders of magnitude through the effects of somatic mutation (1). In addition, the antibody response shows remarkable specificity, so that evidence of any significant amount of cross-reactivity between different antigens is usually taken to indicate close similarity of their structures. The manner in which diversity and specificity operate in the antibody molecule at the level of the three-dimensional structure, as determined by X-ray diffraction, is the subject of this review.

Antibodies were the first members of the immunoglobulin superfamily to be studied structurally. The domain structure observed in antibodies (2–7) has now been seen in many cell-surface proteins that function to control the movement and differentiation of many types of vertebrate cells (8, 9). Members of this family have characteristic domains of approximately 100 amino acids and often contain an internal disulfide loop of 40–70 residues. In those proteins where the three-dimensional structure is known, notably antibodies and the class I MHC antigens (10, 11), the tertiary fold of these domains has been very similar. The recently reported structure of the chaperone protein from *Escherichia coli*, PapD (154), is of particular interest since both domains of this structure have the immunoglobulin fold despite the absence of sequence similarity with the IgG CH2 domains.

Antibodies (see Figure 1) are multivalent molecules made up of light (L) chains of approximately 220 amino acids and of heavy (H) chains of 450–575 amino acids. The light chains contain two immunoglobulin domains; the N-terminal domain is variable, i.e. it varies from antibody to antibody, and the C-terminal domain is constant, i.e. it is the same in light chains of the same type. The heavy chains are made up of an N-terminal variable domain and three or four constant domains. The antibody fragment containing the associated variable domains of the light chain (VL) and of the heavy chain (VH) is called the Fv; the fragment containing the entire light chain and the VH and first constant domain of the heavy chain is called the Fab.

The combining site of antibodies is formed almost entirely by six polypeptide segments, three each from the light and heavy chain variable domains. These segments display variability in sequence as well as in number of residues, and it is this variability that provides the basis of the diversity in the binding characteristics of the different antibodies. These six hypervariable

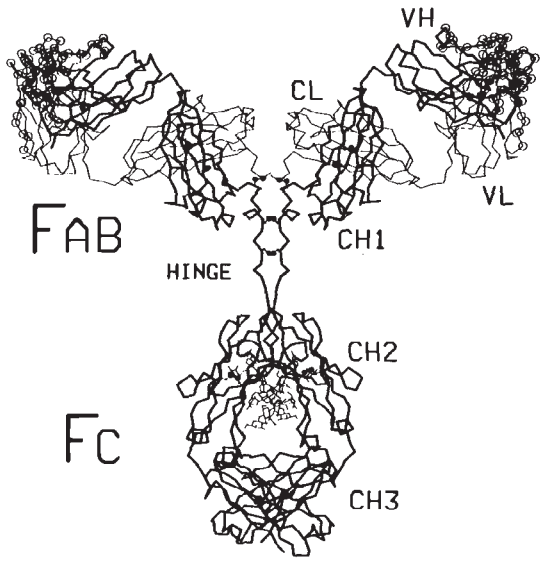

Figure 1 Line drawing of the alpha carbon trace of a model of human IgG1 (E. A. Padlan, unpublished). Thick lines show the heavy chain, thin lines the light chain. The various domains of the light and heavy chains are labeled (VL, VH, CH1, etc). The antigen binding fragment, Fab, and the Fc (consisting of the CH2 and CH3 domains of the two heavy chains) are labeled. The hinge is the heavy chain peptide segment joining the Fab and the Fc. It varies considerably in size in different antibody types. The hinge is often rich in proline and cysteine residues; the latter link the two heavy chains through symmetrical disulfide bonds. These interchain disulfide bonds, as well as those found in the domain interiors, are shown as filled circles. Carbohydrate has been found between the two CH2 domains in human IgG1 Fc (152), and is probably in a similar location in most of the other antibody classes. The two carbohydrate chains are drawn with thin lines.

The molecule was assembled from the Fab of KOL (153), a model of the octapeptide Pro Ala Pro Glu Leu-Leu-Gly-Gly, corresponding to residues 230–237 in the human IgG1 hinge (12), and the human IgG1 Fc of Deisenhofer (152).

segments are also referred to as the complementarity determining regions or CDRs (12).

The three-dimensional structure of antibodies has been the subject of numerous investigations (reviewed in 13–23), and crystal structures for intact immunoglobulins and for a variety of fragments are now available. More than a dozen Fab structures have been determined crystallographically and at least eight of those have been studied with bound ligand. Three Fab-ligand complexes involve small haptens [vitamin K_1OH (24), phosphocholine (25–27), and fluorescein (28)], and five complexes involve proteins [three with hen egg white lysozyme (29–31) and two with influenza virus neuraminidase (32–

34)]. The structures of Fabs with specificities for carbohydrates, DNA, and other molecules have also been determined, although at present only in the uncomplexed form.

The three-dimensional structures of antibodies and of antibody fragments have been reviewed extensively (13–23). Here, we concentrate on the interaction between antibody Fabs and specific ligands. We first consider the binding of antibodies to small molecules (haptens) and then to larger molecules (in particular, proteins). We then analyze the various antibody-ligand complexes of known three-dimensional structure looking at properties that define the specificity and strength of the interactions.

We also discuss briefly how the specificity of antibodies is being utilized for a variety of practical applications. For example, it has long been speculated that antibodies could be used as enzymes. Antibodies and enzymes share the ability for very specific binding. However, antibodies only bind to antigens, while enzymes bind substrates and catalyze their conversion to products. It was proposed 20 years ago by Jencks (35) that antibodies raised against transition-state analogues could have catalytic activity. This possibility has now been demonstrated, and antibody enzymes (abzymes) showing significant activity have been produced (36, 37).

Antibodies have many potential uses in diagnosis and in therapy and, with the advent of hybridoma technology (38), monoclonal antibodies of almost any desired specificity can be produced. However, the monoclonal antibodies that are more easily made are of rodent origin, and the long-term use of these antibodies in human subjects is blocked by the immune response of the host. Attempts have been made to circumvent these difficulties by the creation of chimaeric antibodies containing the constant domains of the host together with rodent variable domains (39–42). This 'humanization' of the more easily available rodent monoclonal antibodies has been further extended by the grafting of the rodent combining site structures to a human framework structure, making feasible the production of essentially human antibodies with designer binding properties (43–45).

For many of these applications a knowledge of the three-dimensional structures of particular combining sites is very helpful, but a crystallographic analysis of each antibody molecule is clearly impractical. The alternative is to use the existing structural information to model the new combining sites. The progress of these model building studies is discussed.

ANTIBODY BINDING TO SMALL MOLECULES

The first Fabs to be analyzed by X-ray diffraction were prepared from myeloma proteins. The antigenic determinants for these antibodies could only be inferred from binding studies using small molecules. Large numbers of

these immunoglobulins with known binding specificities were characterized and sequenced. The first two Fabs whose structures were determined, New, which was shown to bind to a vitamin K_1OH derivative (46), and McPC603, which binds to phosphocholine (47), were analyzed in both the complexed and uncomplexed forms (5, 7, 24–27, 48–50). Until the advent of hybridoma technology, these investigations provided the structural basis for understanding antigen recognition. They are still of considerable interest in showing how high binding and specificity can be produced for relatively small molecules.

In addition to New and McPC603, a third small-molecule-antibody complex, fluorescein bound to 4-4-20, has been recently determined in two laboratories (Ref. 28; M. Whitlow and K. Hardman, personal communication). As the complexes of New and of McPC603 have been reviewed previously (22), we describe them only briefly here.

McPC603 and Phosphocholine

McPC603 will precipitate with pneumococcus C polysaccharide (51), which contains phosphocholine. This precipitation is inhibited by phosphocholine (52), which binds to the McPC603 with a binding constant of 2.0×10^5 per mole (53). X-ray studies show that phosphocholine binds in a pocket in the McPC603 combining site with the choline buried and the phosphate on the surface (25–27). The phosphate group is within hydrogen bond distance of H-Arg52NH1 and H-Tyr330H. There is charge neutralization in that L-Asp91 is at the back of the pocket together with H-Glu35, which is one layer removed from contact with the positively charged choline. At the surface, H-Arg52 and H-Lys54 provide positive charges that complement the charge on the phosphate. The phosphocholine is in contact with only four of the six hypervariable loops: CDR1 of the light chain and all three CDRs of the heavy chain. There appears to be no conformational change upon binding phosphocholine, but this could be partially due to the presence of a sulfate ion from the crystallization medium, which is located in the phosphate-binding position (50).

New and Vitamin K_1OH

New was shown to bind to, among other ligands, the gamma-hydroxy derivative of vitamin K_1 with a binding constant of $1.7 \times 10^5\ M^{-1}$ (46). X-ray studies of the complex of Fab New with vitamin K_1OH revealed that the 2-methyl-1,4-naphthoquinone moiety of the vitamin K_1OH sits in a shallow groove between the heavy and light chains of approximately 16 Å × 7 Å and about 6 Å deep. The phytyl chain of the vitamin K_1OH runs along the surface of the antibody-combining site and contacts a number of residues. At the time of this investigation, the sequence of the New heavy chain had not been determined, so that the interaction between ligand and combining site could

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