Antibody structure and antigen binding

from C. C. F. Blake

ONE of the major problems in immunology has been the reconciliation of the structure of antibodies with the vast range of antigen binding properties they possess. The long and patient study of antibody structure by chemical methods has produced a basic framework for the solution of the problem by showing that antibody molecules have the following features: (1) the molecules are built from two identical heavy chains of ~440 residues and two light chains of ~220 residues, linked together by disulphide bridges; (2) there are four repeating homology regions or domains of ~110 residues in the heavy chains, and two in the light chains; (3) the amino-acid sequence of the N-terminal domains is variable; (4) the sequence in the rest of the molecule is more constant and allows the chains to be characterised as κ and λ light chains which can associate randomly with α, γ and μ heavy chains to give respectively the IgA, IgG and IgM major isotypic classes of antibody molecules; (5) within the variable sequence there are hypervariable regions. This information has been seen to imply that antibody molecules have a common basic structure, in which the antigen binding function is carried by the variable domain whose hypervariable regions are involved in the binding process.

For several years protein crystallographers have been seeking to give this elegant and satisfying model its final structural definition, and at the same time to provide a molecular description of the binding site and its mode of interaction with antigen. As with other studies in this field the work has been hindered by the heterogeneous nature of antibodies and to some extent by the size of the molecule. The former problem has been overcome in the usual way by using myeloma proteins, and the latter, although preliminary X-ray studies on whole immunoglobulins have been reported, by utilising fragments of the molecule; either the papain-induced Fab fragment which contains the binding site, or the naturally occurring Bence-Jones protein, a dimer of the light chains.

In the last year or so, four groups have independently reported high resolution structural analyses of these fragments. Poljak and his colleagues have worked on the Fab fragment of human IgG1 New (Poljak *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3305; 1973; Poljak *et al.*, *ibid.*, **71**, 3440; 1974), Davies and his group on a similar fragment of mouse IgA McPC 603 (Padlan *et al.*, *Nature new Biol.*, **245**, 165; 1973; Segal *et al.*, *Proc. natn. Acad. Sci., U.S.A.*, **71**, 4298; 1974) and the groups led by Edmundson (Schiffer *et al.*, *Biochemistry*, **12**, 4620; 1973) and Huber (Epp *et al.*, *Eur. J. Biochem*, **45**, 513; 1974) have each solved a human Bence-Jones protein. In confirmation of the chemical model all four groups report essentially similar tertiary and quaternary structures.

In the X-ray models the homology domains of ~110 residues appear like beads threaded on a string. Each domain has a tertiary structure that is basically two parallel β sheets between which is the hydrophobic core. The intrachain disulphide bridge, which is a characteristic of each domain, links strands in opposite sheets so that the two sulphurs are located near the centre of the domains. In the constant domain one sheet has four strands and the other three; the variable domain has an additional loop that extends the three-stranded β sheet of the constant domain to five strands. The link between the two domains is made by a short extended chain segment called the 'switch' region.

Each domain associates non-covalently with an equivalent domain in the other chain: the Fab has C_{H1} C_L and V_H V_L dimers, and the Bence-Jones C_LC_L and V_LV_L dimers. This domain dimerisation has two unexpected features. The constant domains associate so that the four-chain sheets are closely opposed and their strands approximately perpendicular, but the variable domains oppose the other β sheet (the one with five strands) which leads to a looser association and a greater intersheet spacing. In the association each domain is related to the other in the pair by pseudo two-fold symmetry, but all four groups have found that the axes of symmetry between the pairs of constant and variable domains are far from colinear; angles of intersection between 120° and 135° are reported. As a result the switch regions bend through different angles in the two chains to give the four domains a tetrahedral arrangement. Although this is not altogether surprising in the Fab where the four domains are chemically distinct, the lack of structural equivalence between the chemically identical chains of the Bence-Jones proteins has lead Schiffer *et al.* to suggest that one of the two light chains in the molecule fulfils the structural role of the heavy chain in Fab. This suggests that Bence-Jones proteins are counterparts of the antigen binding regions of immunoglobulins and may themselves have functional binding sites.

The close similarity of tertiary and quaternary structure observed in the Fab fragments and the Bence-Jones proteins strongly implies the invariance of many features in the architecture of antibody molecules, and allows some important predictions to be made. The amino acid homology between the C_{H2} and C_{H3} domains of the Fc fragment and the C_{H1} domain of Fab makes it highly probable that the X-ray analysis of Fc now under way will reveal an equivalent structural homology. Poljak has shown from his results that the intrachain disulphide bridge in human γ_2, γ_3 γ_2 and μ chains, which is shifted by 83 residues on the heavy chain from its position in the γ_1 heavy chain in his molecule, can be made without altering the observed folding of the polypeptide chains. He also shows that a number of additional or unusual disulphide bridges which have been observed are made between residues that are already in the appropriate orientation. This is additional strong evidence that the results we already have in the Fab parts of the molecule provide the structural basis for all other classes of antibody molecule.

The properties of the antigen binding sites of the two Fab fragments have been investigated by binding their appropriate haptens: vitamin K to Fab New (Amzel *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1427; 1974) and phosphorylcholine to Fab McPC 603 (Segal *et al.*, *ibid.*, **71**, 4298; 1974). In addition Edmundson, following his suggestion of the analogy of Bence-Jones proteins to Fab, has examined the binding of dinitrophenyl ligands to his protein (Edmundson *et al.*, *Biochemistry*, **13**, 3816; 1974). In all cases binding takes place at a cavity between the variable domains, but the cavity has a different size and shape in the different molecules. Amzel *et al.* describe their binding site as a shallow groove 15 Å × 6 Å × 6 Å while Segal *et al.* find a large wedge-shaped cavity 12 Å × 15 Å × 20 Å and Edmundson a conical cavity 10 Å in diameter leading to a deep pocket. The groove or cavity is lined mainly or exclusively with residues from the hypervariable regions of the sequence, which in the structure cluster to form the surface of the binding pocket. Segal *et al.* argue from their results that amino acid substitutions, deletions and additions in these regions could induce such differences in the overall shape, size and general chemical nature of the antigen binding sites that the hypervariability itself could be sufficient structural explanation for antibody diversity. On the other hand neither group working on the Fab fragments has observed any significant conformational change on hapten binding of the kind that might be expected to provide a structural basis for complement fixation or B-cell activation reactions (both functions of the Fc fragment). Nevertheless, Poljak *et al.* do not rule out such a possibility and point to the asymmetric orientations of the switch regions as possible loci of structural alteration, which perhaps takes place when the true antigenic determinant rather than a simple hapten is bound.