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Structure and Specificity of Antibody Molecules

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Structure and specificity of antibody molecules

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The structure of the Fab′ fragment of a human myeloma protein (IgG1 (λ) New) has been determined by X-ray crystallographic analysis to a nominal resolution of 0.2 nm. Each of the structure subunits corresponding to the variable and to the constant homology regions of the light and heavy polypeptide chains contains two irregular β-sheets which are roughly parallel to each other and surround a tightly packed interior of hydrophobic side chains. The regions of the hypervariable sequences in the light and heavy chains occur in close spatial proximity at one end of the molecule, defining the active site of IgG New. The role of these hypervariable regions in defining the size and shape of the active site of different immunoglobulins is discussed on the basis of the three-dimensional model of Fab′ New.

Several ligands that bind to the active centre of IgG New have been used to obtain crystalline ligand-Fab′ New complexes which were investigated by difference Fourier maps. These studies are analysed in terms of the biological function and specificity of antibodies.

Physiological events that take place during the course of an immune response are highly dependent on biological recognition. Immune responses by which vertebrates distinguish self from non-self can be induced by an extremely large number of antigens such as naturally occurring proteins, carbohydrates, nucleic acids and artificially synthesized chemical compounds. Specific recognition is performed by adaptively synthesized antibody molecules capable of neutralizing antigens in complexes of high association constants and high free energy of binding. These complexes involve closely complementary interaction between the antigenic determinants and the 'active' site of the antibody molecule. Following the proposals of the clonal selection theory (Burnett 1959) it is generally accepted that the presence of foreign antigen stimulates the proliferation of pre-existing cells which are capable of synthesizing neutralizing antibodies. Serological characterization and amino acid sequence determinations have shown that antibodies elicited to antigenic determinants (haptens) possess individually unique characteristics associated with their specific recognition function and unique clonal origin. At the same time, antibody molecules share a number of structural features which are a common characteristic of all classes of immunoglobulins (Ig).

Polypeptide chain structure of immunoglobulins

The availability of homogeneous immunoglobulins secreted by cells from human and mice myeloma tumours has greatly facilitated the physical–chemical analysis of immunoglobulin structure. A diagrammatic representation of the structure of a human immunoglobulin of type G1 (IgG1 is the most abundant class in human serum) is shown in figure 1. An IgG immunoglobulin molecule consists of two 'heavy' (H) polypeptide chains of mol. mass 50 000 and two

'light' (L) chains of mol. mass 25000. The H and L chains can be subdivided into 'homology' regions V_L, C_L, V_H, C_H1, C_H2 and C_H3, each containing about 110 amino acid residues.The amino acid sequences of the C_L, C_H1, C_H2 and C_H3 regions are constant or nearly constant and highly homologous to each other. Comparison of V_L and V_H amino acid sequences of different immunoglobulins indicates that the variability of sequences is extremely pronounced in certain 'hypervariable' regions (Wu & Kabat 1970; Kabat & Wu 1971) which in L chains, for example, occur around residues 30, 50 and 95. It has been postulated (Wu & Kabat 1970) that these hypervariable regions of L and H chains contribute to the conformation of the antigen binding site and determine its specificity. Affinity labelling experiments (Singer, Martin & Thorpe 1971; Cebra, Koo & Ray 1974) have indicated that amino acid residues at, or near the hypervariable regions do indeed contribute to the molecular geometry of antigen binding sites.

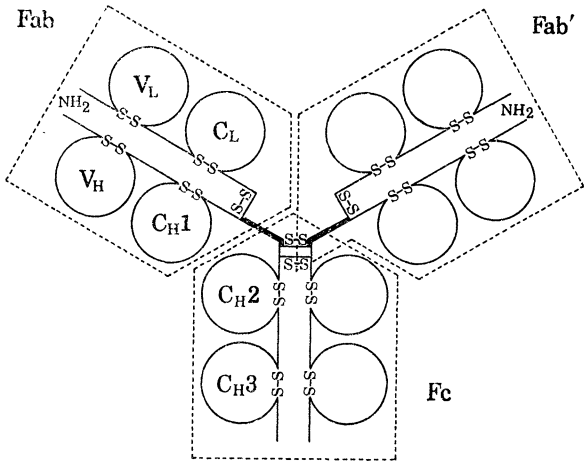

FIGURE 1. Diagrammatic representation of the polypeptide chain structure of a human immunoglobulin (IgG1). The light (L) chains are divided into two homology regions, V_L and C_L, and the heavy (H) chains into four homology regions, V_H, C_H1, C_H2 and C_H3. The thicker trace in the H chain corresponds to the 'hinge' region. The major fragments Fab, Fab' and Fc, the interchain and intrachain disulphide bonds and the N-termini of both chains are indicated. Reproduced from Poljak (1973) with permission from Plenum Press.

Cleavage of an IgG molecule by papain generates Fab fragments (mol. mass 50000) consisting of an L and an Fd polypeptide chain, and Fc fragments (mol. mass 50000) consisting of the C-terminal halves of the two parent H chains. Controlled proteolysis by pepsin followed by reduction of the inter-H chain disulphide bonds generates an Fab' fragment, similar to Fab, consisting of an L and an Fd' polypeptide chain. Both Fab and Fab' retain the antigen binding activity of the parent IgG molecule. The Fc fragment is an effector in biological activities such as complement fixation and attachment to cellular membranes. For further details about immunoglobulin structure and function the reader is referred to more extensive reviews on this subject, such as those of Edelman & Gall (1969), Milstein & Pink (1970), Krause (1970), Hood & Prahl (1971) and Natvig & Kunkel (1973).

THREE-DIMENSIONAL STRUCTURE OF IMMUNOGLOBULINS

X-ray diffraction studies carried out in several laboratories (Sarma, Silverton, Davies & Terry 1971; Padlan *et al.* 1973; Schiffer, Girling, Ely & Edmundson 1973; Epp *et al.* 1974) as

well as our own have made a significant contribution to the study of the three-dimensional structure of immunoglobulins. Results of the low resolution X-ray study of a crystalline Fab′ fragment (Fab′ New from IgG1 (λ) New) and extension of these results to the Fc fragment (Poljak *et al.* 1972) indicated that immunoglobulin molecules are divided into three-dimensional domains. Each of these domains is the site of a physiological property of immunoglobulins: antigen binding ($V_L + V_H$), complement fixation (C_H2), fixation to cellular membranes and passage through placental membranes (C_H3), etc. In subsequent high resolution X-ray diffraction studies (Poljak *et al.* 1973) a Fourier map of the electron density was correlated with the

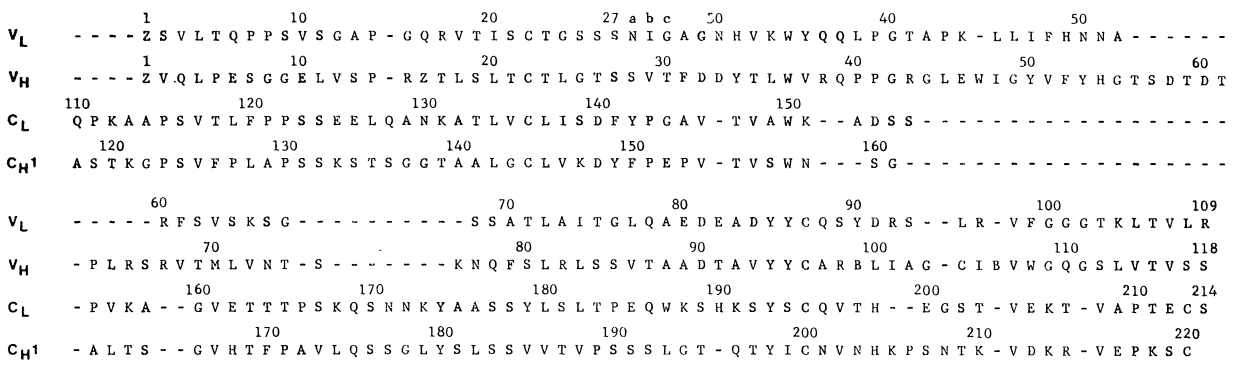

FIGURE 2. Amino acid sequences of the V_L, C_L, V_H and C_H1 homology regions of Fab New aligned by comparison of their three-dimensional structures. The C_H1 sequence is taken from Edelman *et al.* (1969), V_L and C_L from Chen & Poljak (1974) and the tentative sequence of V_H is from Nakashima, Konigsberg, Chen & Poljak (in preparation). Abbreviations for amino acids are as given by Dayhoff (1972).

amino acid sequences of the L and Fd chains (see figure 2) to obtain a three-dimensional model at atomic resolution. A striking feature of this model is that the C_L, C_H1, V_L and V_H homology subunits of Fab′ have a similar three-dimensional folding, in agreement with the homologies of their amino acid sequences and with the postulate of a gene duplication mechanism which gave rise to immunoglobulin genes. The V_L and V_H homology subunits share the basic 'immunoglobulin-fold' of the C_L and C_H1 regions but include an additional length of polypeptide chain (see figure 3) which is unique to the V regions. The V and C homology subunits are predominantly folded in β-pleated sheet conformation. Each homology subunit consists of two irregular β-sheets made of anti-parallel strands of polypeptide chain. These sheets surround a tightly packed interior of hydrophobic side chains which includes the invariant intrachain disulphide bond linking the two β-sheets. About 50 to 60 % of the amino acid residues are included in the β-sheets, giving the appearance of a rigid structural framework.

Variations in the sequence of the V_L, V_H homology subunits occurring outside the hypervariable regions are relatively conservative in nature and compatible with a constant overall three-dimensional structure. Constant residues in these V_L, V_H sequences are involved in important structural features such as internal hydrogen bonds, hairpin bends in the polypeptide chain, interchain and intrachain contacts, etc. Mutations that alter any of these positions, which constitute more than 50 % of the total number of residues, cannot lead to random replacements since they would greatly affect the three-dimensional structure. In contrast, the residues that belong to the regions of hypervariable sequence of the L and H chains occur at one end of the molecule, are fully exposed to the solvent and are not subject to visible structural

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constraints. The overall structural design is such that the exposed hypervariable positions occur at adjacent bends of the tightly packed polypeptide chains. By virtue of this design, different antibody molecules will present at the active site an individually unique conformation of amino acid side chains which are fixed to the constant structural frame of the V_L and V_H subunits.

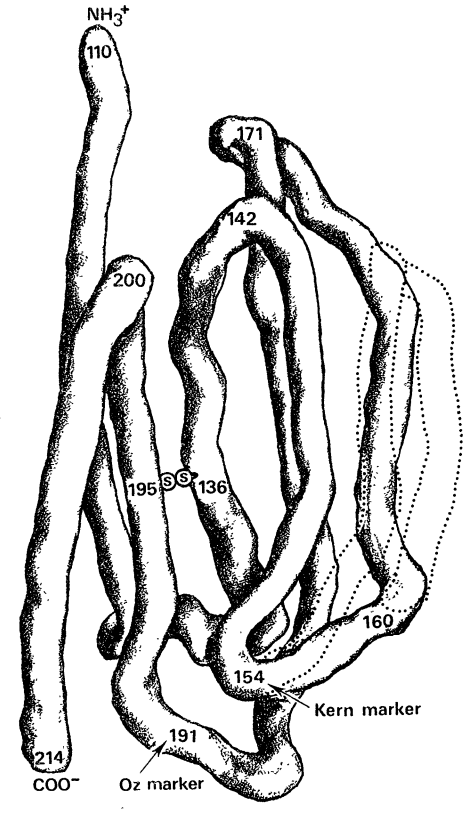

FIGURE 3. Diagram of the basic folding of the polypeptide chain in the homology regions of immunoglobulins. The solid trace shows the folding in the constant regions C_L and C_H. Broken lines indicate the additional loop of polypeptide chain characteristic of the V_L and V_H regions. Numbers designate C_L (λ) residues. Reproduced from Poljak *et al.* (1973).

The patterns of variation and the specificity of some immunoglobulin molecules can be analysed in terms of the structural model presented above. Variations in the amino acid sequences of the hypervariable regions affect the nature of the amino acid residues which are present at the active site, and also the number of such residues. For example, in some human λ chains an insertion of three amino acid residues is found at positions 27a, 27b and 27c, as in the L (λ) chain from IgG New. Since these residues are included in a helical loop their deletion can be easily accommodated without major changes in the path of the polypeptide chain. However, the dimensions of the active site and its contacts with antigens will be modified by this structural variation. Variations in the length of the V_H polypeptide chain are very extensive in the third hypervariable region, around positions 100–110. By virtue of these variations the V_H subunit can play a large role in defining the width and depth of the antigen binding site. Finally, the geometry of the active site can be modulated by different pairings of L and H chains.

Taking the active site of IgG New as a basic structural model it is possible to attempt a correlation between the structure and specificity of the well characterized MOPC 315

anti-2,4-dinitrophenyl (DNP) murine myeloma protein (Eisen, Simms & Potter 1968; Francis, Leslie, Hood & Eisen 1974). The IgA immunoglobulin MOPC 315 is specific for haptenic groups such as DNP and others that include benzene and naphthalene rings. The amino acid sequences of the L and H chains of IgA MOPC 315 and IgG New are highly homologous in the hypervariable regions. The same number of amino acids occur in the first and third hypervariable regions of both L chains and in the hypervariable regions of both H chains. The model

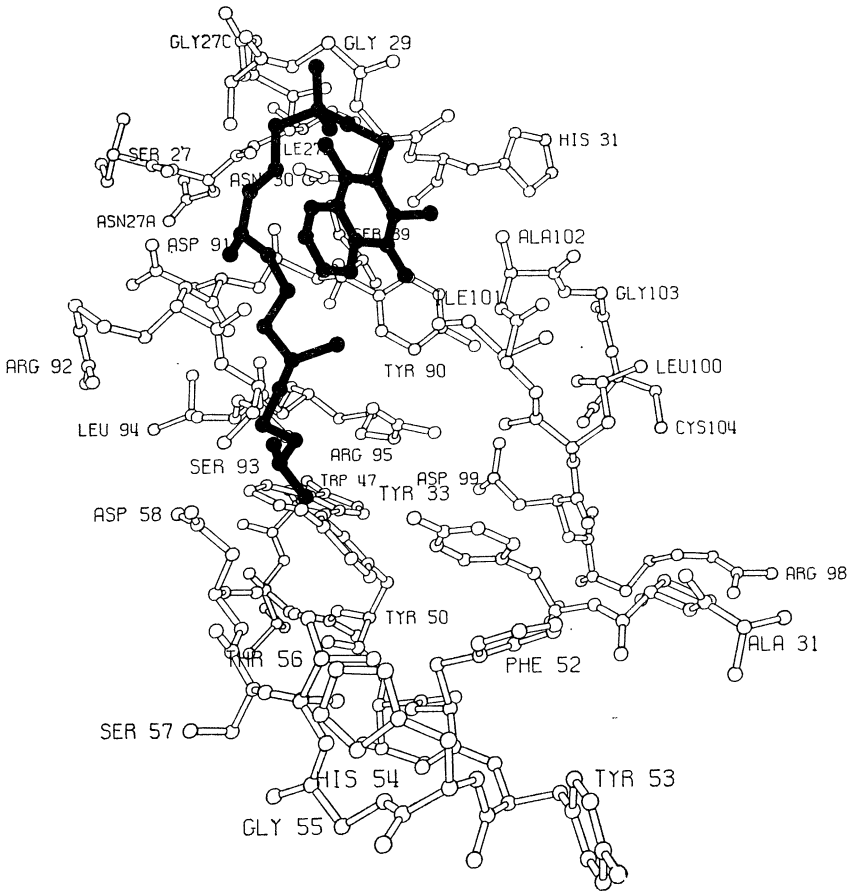

FIGURE 4. View of vit. K_1OH bound at the active site of IgG New. The model of vit. K_1OH was obtained from a 0.35 nm resolution difference Fourier map (Amzel *et al.* 1974). The Fab′ New model was built from a 0.2 nm resolution Fourier map (Poljak *et al.* 1974).

of the IgA MOPC 315 active site that can be built from this comparison (Poljak *et al.* 1974) includes a narrow channel delineated by a very high density of aromatic side chains such as L chain Tyr 34, Trp 98, Phe 99, Phe 103 and H chain Phe 34, Trp 47, Phe 50, Tyr 104 and Phe 105 (the residue numbers are as given in Francis *et al.* 1974). Thus, the high density of aromatic side chains present at this site is in striking correlation with the observed specificity of MOPC 315 IgA for DNP and other aromatic ligands.

STRUCTURE OF A LIGAND–FAB′ COMPLEX

Human immunoglobulins derived from myeloma tumours are capable of reacting with a variety of haptens and antigens, closely resembling the behaviour of induced antibodies

(Eisen, Little, Osterland & Simms 1967; Metzger 1969; Krause 1970). Murine immunoglobulins secreted by plasma cell tumours have also been shown to react specifically with haptens and to exhibit properties similar to those of induced antibodies including the sharing of idiotypic specificities (Potter 1970; Lieberman *et al.* 1974). As a result of these studies it is reasonable to expect that screening of a myeloma protein to search for potential ligands may eventually reveal its antigen binding specificity, or at least its cross reacting specificities.

A screening assay to test for binding activity and possible hapten specificity of IgG New (Varga, Lande & Richards 1974) showed that compounds such as uridine, orceine, acridine, dichlorophenolindophenol, menadione and others bind with a low affinity constant ($K_0 \approx 1 \times 10^3$ L/M) whereas a γ-hydroxy derivative of vitamin K_1 (vit. K_1OH) binds with a higher affinity constant ($K_0 = 1.7 \times 10^5$ L/M). In addition vit. K_1OH binds at a ratio of 2 mol per mole of IgG New which is consistent with the value expected for a hapten–antibody (IgG) system.

Difference Fourier maps at a nominal resolution of 0.6 nm were calculated for each of the crystalline complexes obtained by diffusion of uridine, orceine, dichlorophenolindophenol, menadione and vit. K_1OH into crystals of Fab′ New (Amzel *et al.* 1974). The peaks of highest electron density in these maps were located in close proximity to the regions of hypervariable sequences, at one end of Fab′ New. In view of these encouraging results, a difference Fourier map at 0.35 nm resolution was calculated for the vit. K_1OH–Fab′ New crystalline complex using the native Fab′ New phases obtained for the isomorphous Fab′ New crystals (Amzel *et al.* 1974). In this way, a model of vit. K_1OH was built in which the quinone moiety appears tightly bound at the active site surrounded by the hypervariable regions of the L and H chains (see figure 4). Many close contacts between the naphthoquinone and the phytyl moieties of vit. K_1OH with the polypeptide chain backbone and with side chains present in this region are evident (see figure 4). Menadione, uridine and the other ligands that were studied crystallographically bind at the same site at which the naphthoquinone moiety of vit. K_1OH binds. Binding of ligands to Fab′ New does not induce major conformational changes that can be detected at the resolution limits of the crystallographic studies (Amzel *et al.* 1974).

Discussion

The conformation of the active site of antibodies is determined by regions of hypervariable amino acid sequence, at adjacent loops of the homologous H and L polypeptide chains. By this design a large number of recognition specificities can be incorporated while maintaining the constant overall three-dimensional structure of immunoglobulins. Furthermore, available evidence indicates that different H and L chains can recombine to form immunoglobulin molecules of altered specificity. This permutation could give rise to a large number of specificities with a minimum number of H and L chains. Notwithstanding these features which indicate a potential economy of design we face the conceptual difficulty that the very large number of antibody specificities with which an animal can respond to simple haptens such as (4-hydroxy-3-iodo-5-nitrophenyl)acetyl (Kreth & Williamson 1973) and DNP (Pink & Askonas 1974) requires, under a germ-line gene hypothesis, an unreasonably large gene pool. The size of this pool would be disproportionately large compared to the limited number of genes which are estimated to be present in eukaryotic chromosomes (Judd, Shen & Kaufman 1972; Brenner 1974), about 10^4–10^5 in human chromosomes (Muller 1967; Crow & Kimura 1970).

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In addition, genes in a pool of this size, encoding the extent of diversity apparent in anti-hapten antibodies would be largely repressed and eventually wiped out by natural selection forces following the normal rate of spontaneous deleterious mutations. Of many alternative solutions that have been proposed to deal with this problem and the problem of antibody diversity in general we will consider here (1) somatic diversification by mutation, and (2) the existence of a pool of genes coding for multispecific antibody molecules.

The possible role of somatic mutations as the main source of antibody variability has been widely discussed before (see, for example, Hood & Prahl 1971; Jerne 1971; Weigert, Cesari, Yonkovich & Cohn 1970). We have presented above (see also Poljak *et al.* 1974) structural considerations that place special restrictions on a mechanism of random somatic mutations.

The capacity of individual immunoglobulins to bind different ligands has been taken as support for the postulate that antibodies are multispecific (Eisen *et al.* 1967; Richards & Konigsberg 1973). X-ray crystallographic studies (Amzel *et al.* 1974) have shown that the active site of IgG New can bind a number of ligands (described above) which differ in their chemical properties and that are all bound at the same site. An interesting parallel can be drawn here between IgG New and the MOPC 315 IgA immunoglobulin which has high affinity for DNP and competitively binds other ligands, such as menadione, with high affinity. As pointed out above, the amino acid sequences of IgG New and those of IgA MOPC 315 are highly homologous and contain a similar number of amino acid residues in their hypervariable regions, at the active site. Consequently, a model of the binding site of IgA MOPC 315 can be built based on that determined for IgG New (Poljak *et al.* 1974). In comparing IgG New and IgA 315, we are presented with a close structural and functional homology. The results that have been obtained with both immunoglobulins can be taken as an indication that antibodies are multispecific and can bind different haptens at the same subsite in the active region. The mode of binding, for example by formation of charge transfer complexes involving aromatic side chains at the active site, could be operative for a given chemical class of ligands such as DNP, menadione, flavin mononucleotide, etc. (Michaelides & Eisen 1974). Small conformational changes at the active site could play a significant role in promoting the binding of different haptens to the same antibody molecule.

Independently of the role of somatic mutation mechanisms of diversification, a gene pool encoding multispecific antibodies would be smaller than one encoding monospecific antibodies and would naturally be subject to repeated induction and expression. The advantages conferred to the hosts by such a genetic pool will ensure its maintenance against genetic drift and deleterious mutational events.

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Discussion

S. Lewin (*Department of Postgraduate Molecular Biology, North East London Polytechnic, Romford Road, London E*15)

I am concerned at the conclusion that *no* conformational change accompanies the interaction of the antibody with the antigen (or hapten) since theoretical and experimental considerations (see Lewin 1974) suggest that the reaction of an antibody with its antigen to form the complex is accompanied by some conformational changes.

I wonder whether it is not possible that the conclusion by the speaker that there are no conformational changes could be due to the inability of the experimental techniques used to detect the proportionately small conformational changes involved.

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J. P. Dickinson (*University Department of Radiotherapy, Cookridge Hospital, Leeds, LS*16 6*QB*)

The controversy regarding possible conformational changes in antibody molecules on binding either hapten or antigen is readily resolved on examination of the natures of these two types of substance. Haptens, though able to combine with antibody, are not in themselves immunogenic and do not cause antibody to change its properties (e.g. to allow complement binding), whereas antigens do. It is likely that antigens are at least *bi*-haptenic, and that a correct spatial relation between covalently linked haptens is required to cause conformational changes and changes of function of antibody on binding. The ability to cause changes of function on binding to antibody would seem to be a *sine qua non* of antigenicity, and an important aspect of biological recognition as compared with the purely physicochemical recognition represented by binding of hapten to antibody.

P. J. Sadler (*Department of Chemistry, Birkbeck College, University of London*)

Further to Dr Lewin's question, did you prepare your crystalline hapten–antibody complex by diffusion of the hapten into crystals of the antibody?

R. J. Poljak. Yes.

P. J. Sadler. It seems unlikely that gross conformational changes could occur (without crystal disruption) if the complex is prepared in this way.

R. J. Poljak. Agreed.

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