Comprehensive Immunology 5

Immunoglobulins

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Foreword

Since the discovery more than thirty years ago that antibody activity could be localized to discrete plasma protein fractions, the study of immunoglobulin structure and function has dominated the field of immunochemistry. During this time, sources of homogeneous immunoglobulin molecules have been discovered, the subunit nature of the proteins has been defined, and the three-dimensional structures of the antigen-recognition portion of several antibody molecules have been elucidated. Insights into the complicated genetic control of these proteins are being gained rapidly through analysis of amino acid sequences of naturally occurring and induced homogeneous immunoglobulins. Immunoglobulins have been analyzed by protein chemists as models of complex multimeric systems, examined by geneticists studying serum protein polymorphisms, and employed by molecular biologists as highly selective probes capable of distinguishing minor features of molecular topography. Clinical applications have ranged from the now routine quantitation of immunoglobulin levels to the use of antibodies to detect trace levels of a variety of natural products and drug metabolites. All these applications have depended ultimately on a thorough understanding of the immunoglobulin and its antigen-combining site.

To cover the entire field of immunoglobulin structure and function would require many volumes this size; therefore, subjects presented in this volume represent those which we felt contribute most to our current understanding of this protein family. The first chapters deal with the structure and function of the immunoglobulin molecule. The next group of chapters deals with various aspects of the genetic control of immunoglobulin synthesis, including the evolutionary origins of the immunoglobulins. Additional chapters deal with abnormalities in immunoglobulin structure, the synthesis and secretion of immunoglobulin, and the nature of cell-surface immunoglobulin. Other, related topics will be developed in subsequent volumes of this series devoted to molecular immunology. As can be expected for a multiauthored work, the content of the individual chapters reflects the viewpoint and concern of the authors. While the contributions vary in length and scope, authors, where indicated, have provided references to recent comprehensive reviews to correct apparent deficiencies.

It is our sincere hope that this volume will provide both a background and a source of direction for investigators concerned with the structure, function, and genetic control of immunoglobulins and the role of antibody in host defense.

Gary W. Litman
Robert A. Good

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Studies on the Three-Dimensional Structure of Immunoglobulins

ROBERTO J. POLJAK

1. Introduction

One of the central problems in immunochemistry is that of defining the structural basis for the activity, specificity, and physiological function of antibody molecules. Although several experimental approaches such as amino acid sequence determination and affinity labeling have provided important clues to the solution of this problem, it is generally accepted that X-ray crystallographic analysis is the only technique currently available to reveal the complete three-dimensional structure of proteins. In recent years, several laboratories have succeeded in obtaining atomic-resolution models of immunoglobulins (Ig's) by X-ray diffraction methods. A review of the major conclusions achieved in these studies is the aim of this chapter.

A brief introduction to the polypeptide chain structure of Ig's will be presented first. The reader is referred to other chapters in this volume for a detailed discussion of this topic.

The study of Ig's is greatly facilitated by the occurrence of homogeneous pathological Ig's produced by monoclonal neoplastic lymphocytic cells in mice and in humans. These myeloma proteins, associated with the spontaneous occurrence of multiple myelomatosis and other pathological lymphoproliferative disorders in man and with experimentally induced tumors in mice, have been shown to be closely related to normal Ig's and antibodies by a number of structural and functional properties. Myeloma proteins can be obtained in large quantities and can be readily purified to homogeneous molecular species, thus providing suitable material for detailed structural studies. In general, these myeloma proteins can be isolated as complete molecules, but sometimes only a portion of the molecule is

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present, most frequently the "light (L) chain" of the polypeptide structure (see below). Bence Jones proteins are L chains (isolated from urine) that display a peculiar thermal behavior: they precipitate at 40–60°C, redissolve at 95–100°C, and reprecipitate on cooling.* Ig's can be divided into major classes or isotypes called IgM (macroglobulins, mol. wt. approximately 900,000), IgA (mol. wt. approximately 170,000–500,000), IgG (mol. wt. approximately 150,000), and IgD and IgE (mol. wt. approximately 180,000). In the serum of normal individuals, IgM, IgA, and IgG are found to constitute approximately 5–10, 10–20, and 70–80%, respectively, of the total circulating Ig. These three classes contain carbohydrates that range from 2–3% of the total weight for IgG to about 10–12% for IgA and IgM. The covalently attached carbohydrates are largely hexose and hexosamine with smaller amounts of sialic acid and fucose. IgD and IgE are quantitatively minor components.

The IgG class of Ig's has been the most intensively studied. A diagrammatic structure of a human IgG molecule is shown in Figure 1. The molecule consists of two identical L polypeptide chains (mol. wt. 20,000–25,000) and two identical "heavy" (H) polypeptide chains (mol. wt. 50,000–55,000), which are linked by interchain disulfide bonds to form a covalent arrangement of four chains. Noncovalent interactions between the H and the L chains require the use of drastic conditions (e.g., acid pH, urea) for the separation of this structure into individual polypeptide chain components after reduction of the interchain disulfide bonds. The L chains of human IgG can be antigenically classified into two classes called κ and λ, each characterized by unique sequences in their *C*-terminal regions. Human IgM, IgA, IgD, and IgE also include the same type of L chain (κ or λ), but their H chains are different and are specific to each class.

A major finding in the determination of the multichain structure of IgG was made by Porter (1959), who found that controlled enzymatic digestion of rabbit IgG produces two kinds of fragments, Fab (antigen-binding) and Fc (constant). The Fab fragment (Figure 1, mol. wt. 50,000) retains the antibody activity of the parent molecule, except that it can behave only as a monovalent antibody. No complement-fixation activity can be observed in the immune Fab–antigen complex, indicating that the Fc region is required for complement fixation. Controlled digestion of a human or rabbit IgG protein by pepsin produces a major fragment called $F(ab')_2$ (Nisonoff *et al.*, 1960). By reduction and alkylation of the inter-H-chain disulfide bond(s), the Fab′ fragment (Figure 1) is readily obtained. Fab and Fab′ consist of a complete L chain and a piece (called Fd or Fd′, respectively) that is the *N*-terminal half of the H chain. Human Fd′ is about ten amino acid residues longer than Fd. Similar Fab fragments have been obtained by the use of other proteolytic enzymes such as trypsin. All these proteolytic enzymes split peptide bonds in a region that appears openly accessible and that has been called the "hinge" region ("flexibly") connecting Fab and Fc.

Amino acid sequence studies of the L- and H-chain components of Ig's have shown that these chains possess unique structural features. When the first human myeloma L chains were sequenced, it became clear that L chains of the same class (κ or λ) consist of a *C*-terminal half of constant amino acid sequence and an *N*-terminal half of variable sequence. Because of the possible genetic implications, the

*See Humphrey and Owens (1972) for a detailed review of plasma cell dyscrasias and pathological Ig's.

patterns of variability of L-chain sequences have been extensively analyzed. Thus, it has been observed that within a given class of L chains, there are sequences that are very similar and can be included in one "subgroup." Three subgroups have been recognized in human κ chains and at least four in human λ chains. All chains within a subgroup are very similar in sequence except at certain positions where a pattern of extreme variability is observed (Wu and Kabat, 1970). It is believed that these hypervariable sequences constitute the regions of the L-chain structure that come in contact with antigen, so that the presence of different sequences is correlated with the occurrence of different antibody specificities. Studies on H chains have shown that the region of constant sequence extends to about three quarters of the length of the chain beginning at the *C*-terminus. As in the case of the L chains, the region of variable sequence occurs toward the *N*-terminus of the molecule and spans a length of about 110 amino acid residues. The first H-chain sequences that were determined, in the Fc region of rabbit IgG (Hill *et al.*, 1966), showed another important feature of structure: the existence of sequence homology regions. Two sequences are homologous when they contain chemically related amino acids in the same positions in the polypeptide chain (e.g., serine in the first sequence and threonine at the same position in the second sequence). Another criterion for homology between two sequences is to examine amino acid differences in terms of the minimum mutational events that are necessary to change the

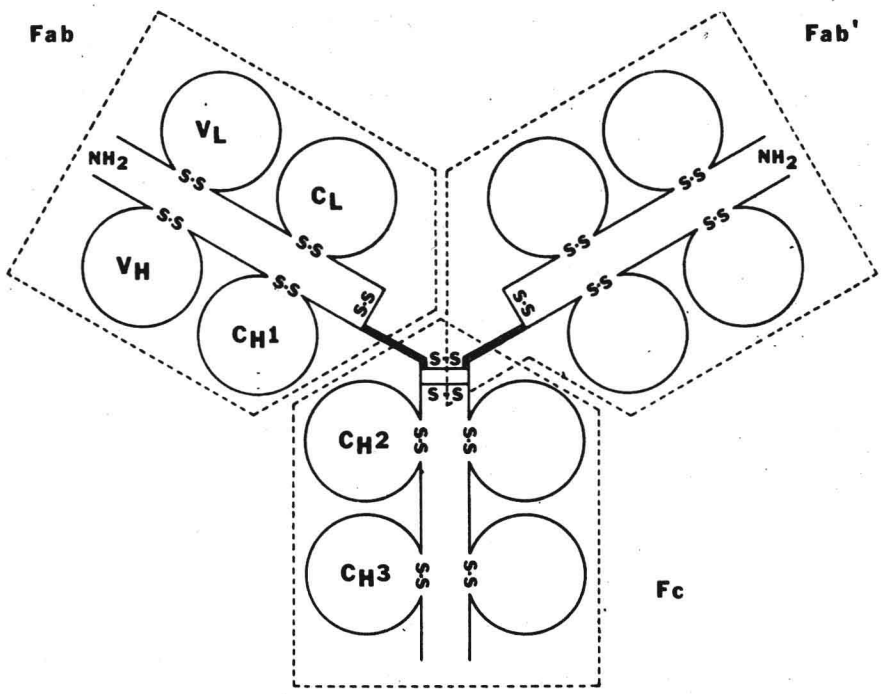

Figure 1. Diagrammatic structure of a human IgG1 molecule. The L chains are divided into two homology regions, V_L (variable) and C_L (constant). The thicker lines in the H chains correspond to the "hinge" region. The four homology regions (V_H, C_H1, C_H2, and C_H3) of the H chains, the interchain and intrachain disulfide bonds, the *N*-terminal regions of both chains, and the major fragments (Fab, Fab', and Fc) are indicated. Reproduced from Poljak (1973) with permission.

nucleotide sequence specifying the first chain to that specifying the second polypeptide chain. If the number of mutations is smaller than can be expected from random chance, then the sequences are said to be homologous. By either of the two criteria mentioned above, one can define four constant "homology regions": C_H1, C_H2, and C_H3 in the H chains and C_L in the L chains (Figure 1). The *N*-terminal, variable regions V_L and V_H (Figure 1) are homologous with each other and have a weaker homology with C_L, C_H1, C_H2, and C_H3. It is interesting to observe that the pattern of a single intrachain disulfide loop of similar length is present in each one of these regions (Figure 1). In addition to their genetic implications, these findings also suggest that the overall three-dimensional folding of IgG molecules is determined by the existence of the homology regions. Inspired by these and other observations, several proposals were made about the folding of the H and L polypeptide chains (Singer *et al.*, 1967; Putnam *et al.*, 1967; Edelman *et al.*, 1969; Welscher, 1969; and others) that can be summarized by describing the tertiary structure of Ig's as consisting of globular "domains," each corresponding to a homology region.

Electron-microscopic (EM) studies have provided the first direct pictures of the general shape and structure of Ig's. The elegant experiments of Valentine and Green (1967), in which a divalent hapten [bis-*N*-dinitrophenyl-(DNP)-octamethylenediamine] was used as a link between several anti-DNP antibodies, provided a picture of the general shape of an IgG molecule and of the arrangement of the Fab and Fc regions (Figure 2). When combined with antigen, the shape is that of the letter Y, with variable separations for the two arms (Fab) of the Y depending on the number of IgG molecules connected by the bis-DNP hapten. The flexibility required to obtain a variable separation is thought to reside in the "hinge" region connecting Fab to Fc. Electron micrographs of an IgA protein produced by the (laboratory-induced) mouse plasma cell tumor MOPC 315 (Green *et al.*, 1971) indicated that the IgA structure consists of globular units or domains. In this study on IgA, a divalent bis-DNP hapten was also used, taking advantage of the fact that the MOPC 315 myeloma protein has the specificity of an anti-DNP antibody. No such globular subunits or domains had been consistently observed in electron micrographs of IgG.

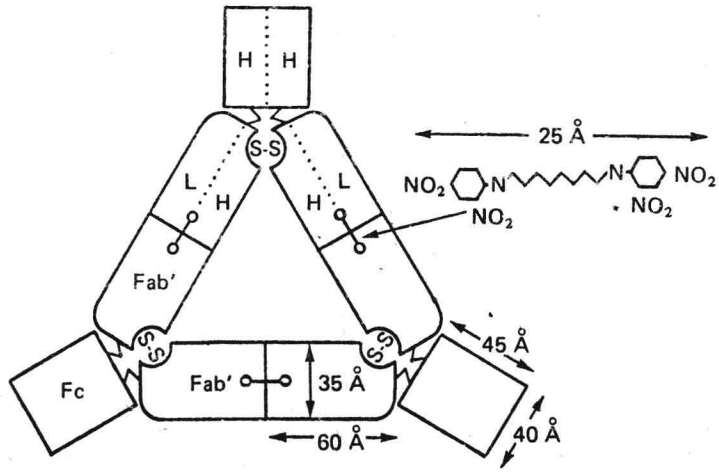

Figure 2. Diagram illustrating a hapten-linked trimer of anti-DNP rabbit IgG antibody molecules. Reproduced from Valentine and Green (1967) with the permission of Academic Press, New York.

Affinity-labeling experiments have contributed to the knowledge of the location and topography of antigen binding sites. In these experiments, a haptenic group is specifically (reversibly) bound and covalently attached to an amino acid side chain on the antibody molecule by means of a chemically reactive group on the hapten. In principle, amino acid side chains that are part of the combining site of antibody molecules or are close to it can be specifically labeled and identified. Using a number of different reactive haptens and antibodies (and also some myeloma proteins that behave like antibodies), a picture had emerged in which the antigen-binding site was defined by the V_L and the V_H regions. Amino acid side chains in, or close to, the regions of hypervariable sequence in L and H chains have been labeled (Singer *et al.*, 1967; Haimovich *et al.*, 1970; Cebra *et al.*, 1971), thus supporting the hypothesis that these regions contribute to (or determine) the antigen-binding site of antibodies. Synthetic antigens have been used as an experimental tool in analyzing the specificity of antibodies, the role of immunodeterminant groups in the antigen–antibody reaction, and the dimensions of the combining sites. With different antigens, the most exposed end of an immunodeterminant group has consistently been found to make the larger contribution to the energy of the binding reaction (Kabat, 1966; Sela, 1969; Schechter, 1971). The dimensions of the binding site have been estimated to be of the order of 35 × 10–15 × 6–10 Å by using antigenic polysaccharides (Kabat, 1966) and polypeptides (Maurer, 1964; Sage *et al.*, 1964; Haber *et al.*, 1967).

2. X-Ray Crystallographic Techniques

Extensive, up-to-date reviews on this subject are available (e.g., Holmes and Blow, 1965; Dickerson, 1964; *Cold Spring Harbor Symposium*, 1971; Matthews, 1976), and the reader is referred to them for a more extensive account of principles and methods. This outline will be a brief, qualitative description of principles and techniques intended for immunologists and immunochemists.

X-ray diffraction has the potential for providing a high-resolution picture of matter in the solid or crystalline state. The basis for this potential is that X rays with wavelengths of the order of magnitude of interatomic distances can be used to obtain diffraction patterns of crystals. There is, however, one major difficulty in producing the desired image of the atomic arrangement: the relative phase of the X-ray waves cannot be measured directly from an X-ray diffraction experiment. Since no X-ray lenses are available, reconstitution of the image from the diffraction spectra must be obtained by the use of a "mathematical lens" function (a Fourier series transformation) in which both the amplitudes and the phases of the diffracted rays are required. The missing phase information can be obtained by a variety of techniques, including "direct methods" in which relationships among the intensities of the diffracted waves are analyzed mathematically to obtain the required phases. For large, complex molecules such as proteins, the only successful and widely used method of determining phases is that of isomorphous heavy-atom substitution. When the phase of each diffracted wave has been determined, amplitudes and phases can be used to calculate a map of the distribution of electron-dense X-ray scatterers (atoms or groups of atoms) that displays their relative densities and positions. In the following sections, some major points of the theory and practice of X-ray diffraction will be considered in more detail.