Antibody Structure: Now in Three Dimensions

The structure of a protein is not completely known until the arrangement of all of its atoms in space has been determined. That goal is now in sight for antibodies, the large, complex proteins that are critical components of the body's defenses against disease. X-ray crystallographers have worked out the three-dimensional structures of portions of four different antibodies from two species. The structures they describe are remarkably similar to one another and indicate that all antibody molecules may fold in the same characteristic way. The results of these investigations confirm the predictions made about antibody structure on the basis of biochemical and immunological studies and should lead to a better understanding of antibody evolution and function.

Crystallographers naturally need crystals to study; normal antibodies, however, can only be isolated as heterogeneous populations of molecules that will not crystallize and would not give usable data even if they did. To get around this problem, the four groups of investigators made use of the fact that certain tumors of plasma cells—the antibody-secreting cells—arise from the multiplication of a single cell and thus produce only one kind of antibody or immunoglobulin molecule, often in large quantities. The tumors, which are called plasmacytomas, may occur in mice and in humans who have multiple myeloma, a cancer of the bone marrow.

Although investigators have not yet been able to obtain crystals suitable for high-resolution x-ray studies of even these homogeneous immunoglobulins, certain portions of the molecules do form good crystals. One such portion is the lighter of the two kinds of polypeptide chains that make up immunoglobulins. Patients with multiple myeloma may secrete large quantities of light chains (known as Bence-Jones proteins) in their urine. Two groups of investigators have performed their x-ray crystallographic studies on human Bence-Jones proteins. One includes Allen Edmundson, Marianne Schiffer, and Kathryn Ely of Argonne National Laboratory and Harold Deutsch of the University of Wisconsin; the other includes Robert Huber and his colleagues at the Max-Planck-Institut für Biochemie in Munich, Germany.

The portion of the antibody molecule named the Fab (for fragment, antigen binding) fragment, because it contains the site that binds the corresponding antigen, may also form the kind of crystals needed for x-ray crystallographic studies. Fab fragments are obtained by enzymatic cleavage of immunoglobulins (Fig. 1). Roberto Poljak and L. M. Amzel of the Johns Hopkins University School of Medicine obtained the Fab fragment they are studying from the blood of a patient with multiple myeloma, whereas a group of investigators at the National Institutes of Health (NIH), including David Davies and Eduardo Padlan of the National Institute of Arthritis, Metabolism, and Digestive Diseases and David Segal of the National Cancer Institute (NCI), obtained theirs from the antibody produced by a mouse plasmacytoma.

The resolutions of the structures obtained by the investigators range from about 3 Å to about 2 Å. This is as good as current techniques—and the quality of the crystals examined—permit. With this degree of resolution, the backbone of the polypeptide chain can be traced and some side chains of the large amino acids can be identified, but each atom cannot be distinguished. For this reason the investigators have had to determine the amino acid sequences of their proteins in order to interpret the x-ray crystallographic data and build molecular models. At NIH, this was done by Michael Potter and Stuart Rudikoff of NCI; Potter provided the antibody being analyzed by Davies and his colleagues.

What is striking about the results of the crystallographic studies is that all of the investigators find almost the same folding pattern in every region or domain of the four antibody components they are studying. As is known from the work of Gerald Edelman of Rockefeller University, Rodney Porter of Oxford University, England, and the numerous other investigators who contributed to the elucidation of the chemical nature of antibody molecules, immunoglobulins of the G class (IgG's) consist of two equivalent heavy chains, with a molecular weight of about 55,000, and two equivalent light chains, with a molecular weight of about 20,000. (The researchers at Johns Hopkins, Argonne National Laboratory, and the Max-Planck-Institut are working with IgG components; those at NIH are studying an immunoglobulin A, or IgA. The overall structure of an IgA resembles that of an IgG but the two classes of immunoglobulins have different types of heavy chains.)

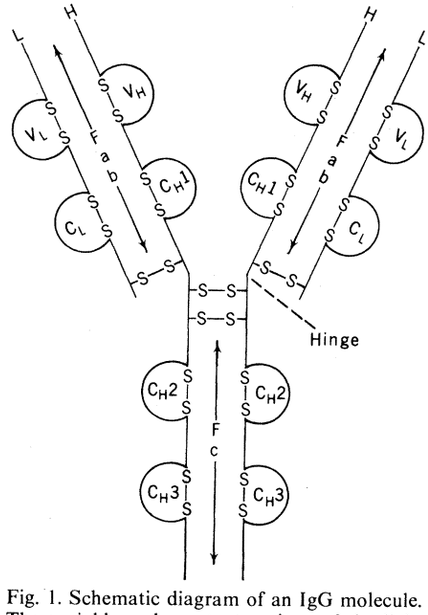

Fig. 1. Schematic diagram of an IgG molecule. The variable and constant regions of the light chain are represented by V_L and C_L, respectively. The variable and constant regions of the heavy chain are represented by V_H and C_H C_H2, and C_H3, respectively. Certain enzymes cleave IgG's in the vicinity of the "hinge" to form two Fab fragments and one Fc fragment.

Each heavy and light chain can be subdivided into domains on the basis of its amino acid sequence (Fig. 1). A light chain has two such domains, one variable and one constant. The amino acid sequence of the variable domain varies from one antibody to another, whereas that of the constant domain is the same for all chains of the same type. An IgG heavy chain has one variable domain and three constant domains. The variable domains confer specificity on the antibody molecule, and two of them—one from the light and one from the heavy chain—form the binding site for antigen. An IgG has two such binding sites. The amino acid sequences of the four constant regions display considerable similarity with one another. Those of the variable domains also have a number of similarities. There is little resemblance between the sequences of the variable and constant domains; however, they all contain approximately 110 amino acids and all have an internal disulfide bridge. Edelman hypothesized that the domains, although having different functions, would have similar three-dimensional structures—and this is what has now been found.

A Fab fragment, which consists of a light chain plus half of a heavy chain, thus contains four domains. In earlier studies, Poljak and Davies each determined the structures of the Fab fragments to a resolution of 6 Å. They found that the fragments measure 40 by 50 by 80 Å and consist of two globular regions of approximately equal size (Fig. 2). One globular region contains the two variable domains and the other the two constant ones, with the four domains arranged in a roughly tetrahedral shape.

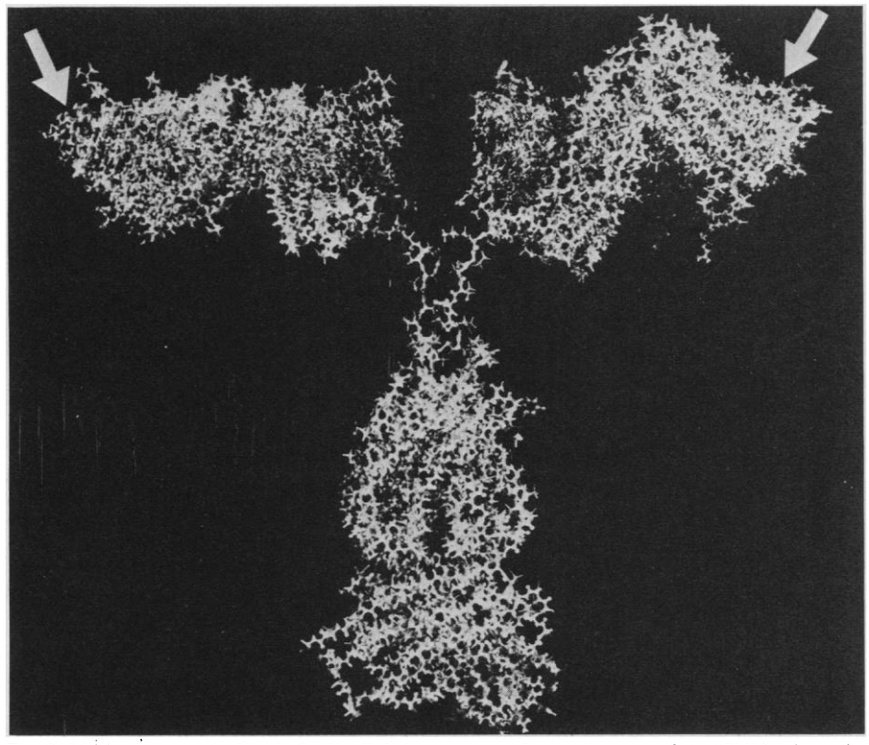

Fig. 2. Molecular model of an IgG. The Fab fragments make up the arms of the "T." Each can be seen to consist of two globular regions. The arrows show the part of the antibody that binds antigen. The Fc fragment constitutes the leg of the "T." Since crystals suitable for determining the three-dimensional structure of this portion of the immunoglobulin molecule have not yet been obtained, the model was constructed by combining information about the known sequence of an IgG heavy chain with the newly determined three-dimensional structures of the constant domains in the Fab portions of the molecule. [Source: David Davies and Eduardo Padlan, National Institute of Arthritis, Metabolism, and Digestive Diseases]

The higher-resolution studies revealed that both variable and constant domains are cylindrical and that the antiparallel β-pleated sheet is the predominant structural feature of both (Fig. 3). In this kind of structure the backbone of the polypeptide chain is extended and the neighboring chains run in opposite directions. The amino acid side chains are oriented at right angles to the direction of the polypeptide chain, with adjacent side chains appearing on opposite sides of the backbone.

In each domain two layers of pleated sheet fold into a sandwich-like structure. One layer is composed of a four-segment sheet and the other has three segments. The disulfide bond is located between the layers and connects the same segment of the four-chain layer of all domains to the same segment of the three-chain layer. Hydrophobic side groups flank the disulfide bond and fill the interior of each domain. Variable domains usually have an additional loop that is not found in constant domains and is not part of the sandwich layers.

Despite the similarities in the three-dimensional structures of the variable and constant domains, there is a major difference in the way the variable domains associate compared to the way the constant regions do. The former are in contact through their three-segment surfaces, whereas the latter associate through their four-segment layers. This requires that the constant domains rotate about 165° with regard to the variable domains. There is also a difference in the way the two chains of the Fab fragments are bent. Both bend in the area between domains but the heavy chain bends more than the light one.

Although the Bence-Jones protein that Edmundson and his colleagues are studying is equivalent to a single light chain, they found that it crystallized as a dimer. Moreover, the dimer looks like a Fab fragment since one of the chains assumes the same conformation as the heavy chain does. This is surprising because the amino acid sequence of a polypeptide determines its conformation, and yet here is a situation in which two chains with identical sequences have different three-dimensional structures.

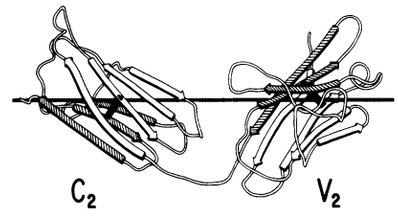

Fig. 3. Schematic diagram of the three-dimensional structure of a human Bence-Jones protein. The arrows represent the amino acid sequences forming the antiparallel β-pleated sheets. The dark bars represent the internal disulfide bonds in the constant (C) and variable (V) domains. [Source: Allen Edmundson, Argonne National Laboratory]

Another important question about antibody structure that the x-ray crystallographers have answered concerns the nature of the site that combines with antigen. It was known that the variable domains formed the site and that certain segments of amino acid sequences in these domains were more variable than others. These were called hypervariable regions by Elvin Kabat of Columbia University Medical School and Tai Te Wu, now at Northwestern University, who found that they generally center around amino acid residues 20, 50, and 90 (as counted from the end of the polypeptide chain that has the free amino group). On the basis of their immunological and chemical studies, these investigators predicted that the hypervariable regions formed the antigen-binding site—and the current studies have now confirmed this prediction. They show that the variable regions of the light and heavy chains fold and associate in such a way that the hypervariable regions are brought together to form a fairly large antigen-binding surface. The hypervariable regions are largely outside the regions constituting the pleated sheet framework of the domains.

The investigators were aided in their analysis of the antigen-binding sites by the identification of small molecules or haptens that bind to them. Haptens, when complexed to large molecules such as a protein, will elicit the production of specific antibodies. The Bence-Jones dimer acts like an antibody in that it too will bind haptens.

The materials studied thus far have antigen-binding sites of different shapes. In the Fab fragment studied by Poljak and his colleagues, the site is a shallow groove. In the one studied by the NIH investigators, it is a wedge-shaped cleft. And the Bence-Jones dimer has a conical site that terminates in a bulb-shaped pocket.

None of the investigators observed a change in the conformation of their materials as a consequence of hapten binding. Such a change might be expected because antigen binding to the variable domains in effect turns on certain activities of the antibody molecule that are thought to be functions of the constant domains. The investigators point out that these experiments do not rule out the possibility of such a change in shape. The haptens they use are small

(Continued on page 1114)

RESEARCH NEWS

(Continued from page 1076)

(phosphorylcholine at NIH, a derivative of vitamin K at Johns Hopkins, and 2,4-dinitrophenyl groups at Argonne) compared to ordinary antigens, and they interact with only a few residues in the combining site. They might miss the ones involved in triggering conformation changes. Furthermore, the antibodies were studied in the crystalline state, and the results may not be applicable to what happens when the proteins are in solution.

At least one group of investigators, including I. Z. Steinberg and J. Schlessinger of the Weizmann Institute of Science in Rehovot, Israel, has evidence that antibodies in solution undergo a conformation change when they bind antigen. They determined the effect of antigen binding on the circular polarization of fluorescence of antibodies. The investigators observed changes only with large antigens and not with phosphorylcholine.

The picture of antibody structure emerging from all this is one in which certain segments of both variable and constant domains form a structural framework that has changed little throughout the course of antibody evolution. Several investigators pointed out that the resemblances in the three-dimensional structures of the different domains support the hypothesis that they all originated from duplication of a single primordial gene. When changes in amino acid sequences did occur in the framework regions, they were such as to not markedly disturb the basic folding pattern. On the other hand, alterations outside of this framework, for example, in the hypervariable regions of variable domains, can give rise to antibodies with different specificities. Alterations in the nonframework sequences of constant domains would permit the evolution of domains capable of performing different functions.

Because of the similarity between the Bence-Jones dimer and the Fab fragments, Edmundson thinks that the dimer may represent a prototype for a primitive antibody, and a possible intermediate in the evolution of the four-chain immunoglobulin molecule. He suggests that the rotation of the constant domain relative to the variable one was a critical step in the evolutionary process because it means that different amino acid residues would be needed for maintaining the association of each domain pair. Those not involved in the interaction would necessarily also be different and hence the domains could evolve to perform different functions. The eventual result would be immunoglobulins with the structures and functions that we know today.—JEAN L. MARX

Personnel Placement

POSITIONS WANTED

Biochemical Endocrinologist, 2-year postdoctoral experience in mechanism of hormone action, receptor binding, feedback regulation, enzyme induction and RIA. Seeks research, teaching position. Box 411, SCIENCE. 9/26; 10/3, 10

U.S. **Foreign Graduate,** M.S. with research and clinical experience in bacteriology, virology, parasitology, biochemistry, epidemiology, rural health delivery, seeks position in environmental health, preventive medicine, related field. Box 412, SCIENCE. X

Immunologist—Biochemist—Microbiologist: Ph.D. (immunology-immunochemistry); M.S. (microbiology); B.S. (chemistry). More than 12 years of postdoctoral research experience: antigens isolation—purification; antibody production—characterization; antigen-antibody interactions and assays (including radioimmunoassays); various immune responses and immunological-immunochemical techniques in both in vitro and in vivo systems; enzymes and membrane receptors; protein-protein and protein-drug bindings and interactions; protein chemistry and biochemical methods. Also, college teaching experience, radioisotopes license and publications. Desires position in research, teaching/research, research/teaching. Available immediately. P.O. Box 58, Rancocas, New Jersey 08073. X

M.D./Ph.D. (1976). **Neuroscientist.** Publications. Desires research/teaching position in university, industry, or government. Available summer 1976. E. E. Fahringer, Department of Physiology, University of Pittsburgh, Pittsburgh, Pa. 15261. X

Postdoctoral Research Fellowship: Ph.D. December 1975. Publications. Sound knowledge: spectroscopy and synthetic methods. Experience in pharmaceutical industry. Languages: English, French, and German. Seeks fellowship in chemistry or pharmaceutical department. Box 413, SCIENCE. X

Zoologist. Ph.D. Desires teaching position. Interests include invertebrate zoology, limnology, aquatic microbiology, parasitology, embryology, comparative anatomy. Dr. Wilson, Route 2, Box 575, Russell Springs, Kentucky 42642. X

POSITIONS OPEN

ASSISTANT PROFESSOR. We are accepting applications until 1 November for position of assistant professor in a research-oriented Biology Department to team teach in the area of animal physiology at the graduate and undergraduate levels. Candidates must be interested in developing a dynamic research program and will work with two other comparative animal physiologists in modern and well-equipped integrated laboratories with access to aquatic holding facilities. Ability to teach in French is an asset, failing which, willingness to learn is essential. Applications including curriculum vitae, copies of representative publications, a research proposal, and names and addresses of three referees should be sent to **Professor G. Kaplan, Chairman, Department of Biology, University of Ottawa, Ottawa, Ontario K1N 6N5, Canada.**

ASSOCIATE PROFESSOR OR PROFESSOR
Department of Obstetrics and Gynecology

The University of Michigan Medical School is seeking an M.D. who has had major administrative responsibility in addition to experience in managing high risk obstetric service. To direct large outpatient service and assume major consultant role in expanding perinatal program. Evidence of academic achievement essential. Code #300FO. Send résumè to **Box 416, SCIENCE.** *A Nondiscriminatory, Affirmative Action Employer.*