

Ann. Rev. Immunol. 1983. 1:87-117 Copyright © 1983 by Annual Reviews Inc. All rights reserved

STRUCTURAL BASIS OF ANTIBODY FUNCTION

David R. Davies and Henry Metzger

National Institute of Arthritis, Diabetes, Digestive, and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205

INTRODUCTION

It is less than 20 years since the general architecture of antibodies was elucidated and even less time since the explicit molecular basis of antibody specifi

about the immune system, two basic principles have emerged: (a) Antibodies remain the only known structures whose diversity is sufficient to explain the fine specificity exhibited by the immune response; and, (b) antibody function is mediated by a molecule whose structure consists of two distinct regions—one that carries a recognition site for antigenic determinants, and a second by which the antibody reacts with receptors of a variety of effector systems.

In this review we examine the current information on the structure of antibodies. We do not describe again the basic four-chain structure of immunoglobulins nor the division into variable and constant regions, which are by now well known (e.g. 4, 90, 118). We instead concentrate on the higher resolution data, much of which is still in the course of refinement. We discuss the Fabs, in particular with reference to the combining site and the specifi

binding site of protein A of Staphylococcus aureus and of Clq; and the structure of the hinge with reference to its possible role in separating Fab

Whereas the characterization of the structures of individual proteins and



88 DAVIES & METZGER

sophistication, the interaction of two or more macromolecules still presents considerable difficulties. It is not surprising therefore that our understanding of how antibodies interact with the macromolecular receptors on effector systems (e.g. Clq in the complement pathway, Fc receptors on cell membranes) is much less advanced. Our review of this aspect of antibody structure and function therefore involves more questions than answers.

IMMUNOGLOBULIN STRUCTURE

General Comments

Our knowledge of the three-dimensional structure of antibodies at atomic resolution rests mainly on X-ray diffraction investigations of fragments. Intact proteins for which X-ray analyses have been carried out consist of Kol (106), an IgGl(λ) human myeloma protein, the protein Dob (161), an IgGl(κ) human cryoglobulin, and recently the human myeloma IgGl(λ) protein, Mcg (129). In Kol, and also apparently in another immunoglobulin, Zie (58a), the crystal structure contains an unusual feature: The Fc occupies a number of different positions in the crystal that are not crystallographically related, with the result that no significant electron density occurs in this region of the crystal, there being an abrupt drop in density at the end of the hinge. In both Dob and Mcg there is a 15-amino-acid-residue deletion in the hinge (63, 169), thus, presumably, reducing the flexibility of the molecule and enabling the Fc to be located in the electron density, although in the case of Dob the crystals are disordered and do not diffract to high resolution.

The structures of three Fabs have been published, Newm (148), Kol (106), and McPC603 (152), as well as the structures of a number of V_L dimers and L chain dimers (4). The structure of human IgG Fc has been determined, and also its complex with protein A of *Staphylococcus aureus* (43). These structures have been reviewed most recently by Amzel & Poljak (4) and are not covered comprehensively in this review.

Because of the deficiencies in the crystals of the intact molecules, our knowledge of the whole antibody molecule has to be a sum of its parts. The flexibility of the molecule, in particular in the region between the Fab and the Fc, may preclude for some time visualizing directly by X-ray diffraction an intact molecule with intact hinge at atomic resolution. However, the checks that can be made on this composite three-dimensional picture of the antibody molecule are reassuring. Thus, the Kol Fab in isolated form in the crystal is quite similar to the Fab of the whole molecule in its crystal form. Also, the Fc in Dob has, within the experimental error of the comparison, the same overall structure as does the Fc in the isolated Fc crystals.



Fab Structure and the Antibody Combining Site

McPC603 AND THE PHOSPHOCHOLINE BINDING SITE The structure of McPC603 Fab, a mouse myeloma IgA (k) with phosphocholine (PC) binding capability, has been determined at 3.1-Å resolution (41, 42, 125, 152) and is being refined to 2.7 Å (Y. Satow, D. R. Davies, manuscript in preparation). The overall three-dimensional structure of the Fab is illustrated in Figure 1, which demonstrates the strong lateral association between domains of the light (L) and heavy (H) chains, together with the relatively weak longitudinal interactions along each chain. Figure 1 also shows the clustering of six of the seven hypervariable regions at the tip of the Fab, forming the complementarity-determining surface (89, 90). The variable domains have a very similar three-dimensional structure for both the L and H chains and across species (4, 42, 123). The constant domains C_L and C_H1 are also very similar. Both the variable and the constant pairs of domains are related by approximately twofold (rotation about the V axis of 180° will superpose V_L on V_H) and the angle between the two axes has been referred to as the elbow bend of the Fab and has been observed to vary

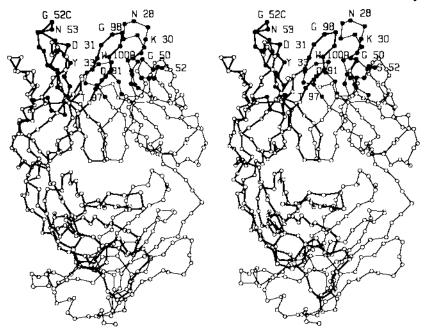


Figure 1 The α carbon backbone of McPC603 Fab. The heavy chain is represented by the thick line. The two variable domains are at the top and the constant domains are at the bottom of the figure. The complementarity determining residues (CDR) are shown as filled circles. Two residues in each CDR loop have been labeled.



between approximately 137° for Fab Newm (4), 135° for McPC603 (152), 147° for Dob (161), and approximately 170° for Kol (106).

Figure 2 shows the combining site of McPC603 with PC bound. (Y. Satow, E. A. Padlan, G. H. Cohen, D. R. Davies, manuscript in preparation). The choline is attached at the bottom of a pocket located principally between the hypervariable regions H3 and L3. The phosphate is on the surface and contacts residues from the heavy chain. It is apparent that PC is a small molecule and that the greater part of the hypervariable surface is not directly in contact with it. At the front of the pocket there are two hydrogen bond donors positioned within reasonable hydrogen bonding distance of the phosphate oxygens; these are the hydroxyl group of Tyr 33H and the guanidinium group of Arg 52H (152). The residues lining the inside of the pocket are Tyr 94L on the right side, Asp 91L on the left, Leu 96L at the back, (125, 146), and the side chain of Trp 100aH at the top left. In addition, the backbone of residues 92–94L form the lower rim of the front of the pocket.

CONFORMATIONAL CHANGE One of the mechanisms proposed for effector function activation involves an allosteric change upon antigen binding (110). Since crystals of immunoglobulins have large solvent channels and can bind to haptens soaked in through these channels, crystallographic investigation offers a direct way for observing conformational changes, when they occur.

When PC binds to McPC603 in the crystal, no significant conformational change occurs in the protein. There is a small movement of Trp 104a away

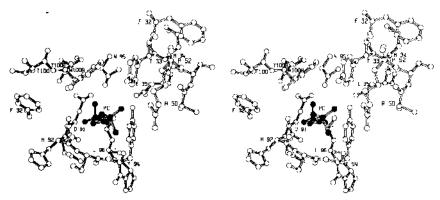


Figure 2 Stereo drawing of the combining site of McPC603 with phosphocholine bound. The lower residues (91 96 and F32) are from the light chain. The remaining residues belong to the three complementarity determining regions of the heavy chain. The phosphocholine has the phosphate group in front with the choline moiety buried in a pocket.



from the pocket, but no other change of any significance. However, there are several reasons why it cannot be concluded from this observation that antigen-antibody interaction results in no conformation change:

- 1. The crystals are grown in a concentrated ammonium sulfate solution, and it has been observed that in the absence of PC there is a peak at the phosphate binding site interpreted to be a sulfate ion (126). A conformational change might have been triggered by the presence of this sulfate ion, so that no additional change would be observed upon PC binding. However, in this respect it should be noted that in Fab Newm, (148) no conformational change occurs upon binding of a neutral vitamin K_1 derivative.
- 2. PC is small and the association constant ($\sim 10^5$ M ¹) with McPC603 might be insufficient to trigger a conformational change that could be induced by a larger, more tightly binding antigen. The same consideration applies to Fab Newm.
- 3. The only two structures at atomic resolution of immunoglobulins with known binding specifi

rule out the possibility that changes that occur with the intact molecule might not occur with fragments (81).

Thus, although there is no support from X-ray diffraction for a conformational change associated with antigen binding, such a change cannot be rigorously excluded.

McPC603: THE CONTACTING RESIDUES AND THE EFFECT OF CHANGES IN THE COMBINING SITE The PC molecule is in direct contact with only a limited number of residues. They include side chains from all three heavy chain hypervariable regions and from one (L3) light chain region. The next most distant range of contacts contain many residues that play a role in positioning the directly contacting residues and changes in these might be expected to influence PC binding. An example of such a side chain is Glu 35H, a residue in the interface between V_H and V_L that makes a hydrogen bond with the hydroxyl of Tyr 94L, which is in turn a major contacting residue with hapten. A mutant of S107, a PC-binding myeloma protein, has been observed that has lost the ability to bind PC and also that fails to agglutinate PC-SRBC (144). Amino acid analysis showed that the mutation results in substitution of an alanine for glutamic acid in position 35H. Although a change of this magnitude is likely to produce a significant rearrangement of side chains in its vicinity simply because of the difference in volume of the two side chains, the loss of contact with Tyr 94L reduces an important constraint on a residue in direct contact with hapten.

Another mutant observed by Cook et al (38) is more puzzling. The



DOCKET

Explore Litigation Insights



Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time** alerts and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.

