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The Three-Dimensional Structure of a Phosphorylcholine-Binding Mouse Immunoglobulin Fab and the Nature of the Antigen Binding Site

(x-ray diffraction/domain structure/hypervariable cavity/hapten binding/antibody diversity)

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ABSTRACT **The structure of the Fab of McPC 603, a mouse myeloma protein with phosphorylcholine binding activity, has been determined to 3.1-Å resolution. The four domains are found to be structurally similar with a well-defined double-layer structure. A large cavity exists at one end of the fragment, the walls of which are formed exclusively of hypervariable residues. Phosphorylcholine binds in this cavity and forms specific interactions with several well-defined amino-acid side chains of the protein. The hapten is bound asymmetrically and interacts more with the heavy chain than with the light chain.**

We have been investigating the nature of antibody–antigen interactions by means of a crystallographic investigation of the Fab fragments of mouse myeloma proteins with known binding specificity (1, 2). The testing of myeloma proteins in mice for antigen-binding activity has revealed that many of them interact by precipitation, agglutination, or complement-fixation with common natural antigens from the environment of the mouse (3, 4). Immunochemical analysis of myeloma protein–antigen interactions has in many cases led to the identification of the chemical group (hapten) on the antigen molecule.

Antibodies, induced to dextran, levan, and phosphorylcholine in mice, share cross-specific antigenic (idiotypic) determinants with myeloma proteins of corresponding specificity (5, 6; M. Potter and R. Lieberman, unpublished observations). These findings strongly suggest the close relationship of antigen-binding myeloma proteins in mice to natural or induced antibodies. One of these myeloma proteins, from McPC 603, precipitates with antigens from several pathogenic organisms, and has been demonstrated to bind specifically to phosphorylcholine (3). We have previously reported crystallization of the Fab fragment of McPC 603 protein (1) and its structure at 4.5-Å resolution together with the location of its phosphorylcholine-binding site (2). Here we present the results of a 3.1-Å structure determination and the molecular details of phosphorylcholine binding. We compare these results with the crystallographic results on a human Fab fragment with hydroxyvitamin K_1 binding properties (7, 8), and with two human Bence-Jones proteins (9, 10).

MATERIALS AND METHODS

The preparation and crystallization of McPC 603 pepsin Fab has been described (1). The crystals used were obtained from 42% saturated ammonium sulfate solutions, 0.1 M imidazole (pH 7.0). They belong to the space group $P6_3$ with a = b = 162.5, c = 60.8 Å. Heavy atom derivatives were prepared by soaking the crystals in solutions containing $TmCl_3$, $K_2Pt(CNS)_6$, iodine, and combinations of these (E. A. Padlan, D. M. Segal, G. H. Cohen, T. Spande, and D. R. Davies, in preparation). Approximately 11,000 reflections (mean figure of merit 0.73) were used to calculate a 3.1-Å electron density map. Details of the crystallographic analysis are being published elsewhere. A Kendrew skeletal model of the entire Fab has been constructed with the aid of the amino-acid sequence, which is for the most part known. An optical comparator of the type proposed by Richards (11), but with the mirror parallel to the electron density sections, was used to facilitate the model building. Coordinates have been measured for the variable region of the molecule and improved by the Diamond model-building program (12). Hapten binding has been studied by difference-Fourier techniques using data from crystals of McPC 603 Fab soaked in saturating concentrations of phosphorylcholine as described (2), but here extended to 3.1-Å resolution.

The amino-acid sequence of the variable portion of the heavy chain (V_H) is completely known (Table 1). Approximately half the C_H1 region sequence has been determined (Rudikoff, unpublished), and a substantial part of the remainder may be surmised from its expected homology with MOPC 315 (13). The V_L sequence is mostly known and its determination is being actively pursued. The constant portion of the light chain (C_L) is assumed to be identical with the sequence of the C region of other murine K light chains (14, 15). Light chain hypervariable regions were assigned according to Wu and Kabat (16). In the heavy chain these regions were identified by comparison with other heavy chain sequences, with deletions and insertions introduced to maximize homology (17).

RESULTS

The electron density map is clear enough to permit tracing the course of the polypeptide chain with confidence. While many of the smaller side groups are lost in the background noise, particularly those on the exterior of the molecule, the larger aromatic side chains are for the most part clearly visible, and serve as markers in fitting the amino-acid sequence to the map. Although the four disulfide bridges were located, they do not exhibit the highest peak density in each domain.

Overall Structure. The overall appearance of the IgA(K) 603 Fab is very similar to that previously observed in the human

Abbreviations: C_H1 and V_H; C_L and V_L signify the constant and variable domains of the heavy (H) and light (L) chains, respectively, of the Fab. H1, H2, and H3; L1, L2, and L3 are the first, second, and third hypervariable regions of the H and L chains, respectively.

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TABLE 1. *Variable region sequence of McPC 603**

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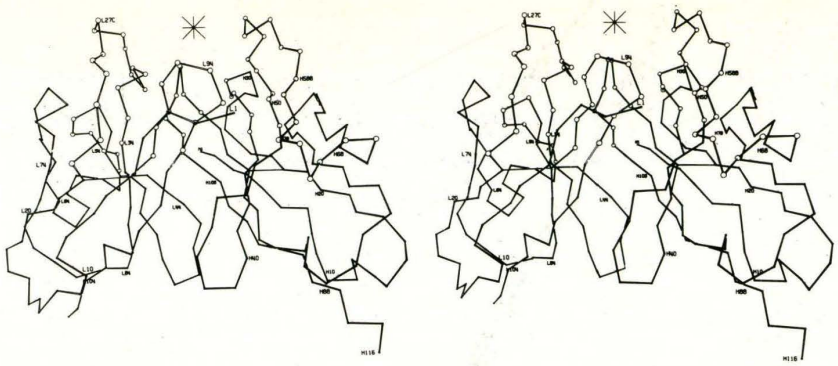

FIG. 2. Stereo drawing of the α-carbon skeleton of the variable region. Open lines represent the light chain and solid lines the heavy chain. The hypervariable residues are represented by the large open circles. The star marks the location of the large hypervariable cavity.

tion, this heavy chain interdomain interaction involves no charged amino-acid side chains.

It is apparent that the dominant force maintaining the structural integrity of the McPC 603 Fab is due to the strong lateral interactions between homologous domains (V_H–V_L; C_H1–C_L) rather than the much weaker longitudinal interactions between domains of the same chain. The interactions between the two constant domains occurs principally between the layers containing segments S1, S2, S7, and S6. The interaction between the variable domains is quite different, and involves the segments S3, S8, and S9, as well as a major portion of the extra loops S4-S5. Additional intervariable domain contacts occur between parts of the hypervariable loops, especially in the regions B23 of the L chain and B89 of the H chain.

The constant region is more compact than the variable region, probably as a result of having fewer bulky residues in the interface between C_L and C_H1. The interacting layers in this region are about 10 Å apart; this results in a distance between the two S-S bridges of 18 Å. In the absence of complete sequence information for C_H1, the interactions across the C region interface cannot be determined with confidence.

In the variable region the interacting segments are 12–15 Å apart, and this greater separation is reflected in the distance between the intervariable domain disulfides of approximately 25 Å. In spite of our lack of complete light chain sequence, many of the interdomain contact residues can be identified. They span the complete range of variability. Some, for example, Leu 45 and Trp 47, have been found in all heavy chain sequences, and Gln 39 has been found in most (17, 19). Similarly in the light chain, Tyr 36, which makes contact across the interface, is the residue most frequently found in this position in kappa chains (19). In addition, many of the interacting residues are found in the hypervariable regions. Indeed, the large loop produced by the six-residue insertion in L1 is in intimate contact with most of the residues in H3. Moreover, some residues in L3 interact with residues in both hypervariable and nonhypervariable portions of the V_H domain.

The Antigen-Binding Site. The 4.5-Å investigation revealed the location of the hapten-binding site (2). The appearance of two peaks on the difference electron density map led to the suggestion that, in the native crystals, the phosphate binding site was occupied by a sulfate ion. The present native electron density map at 3.1-Å resolution shows a large peak, tetrahedral in appearance, and making contact with side chains, in the phosphate position. The choline site is revealed by a 3.1-Å difference map between native and phosphorylcholine-soaked crystals.

The site of hapten binding is in a large wedge-shaped cavity, which exists at the amino-terminal end of the molecule (Fig. 2). The cavity is approximately 12 -Å deep, 15-Å wide at the mouth, and 20-Å long, the walls of which are lined exclusively with hypervariable residues (16). Only five of the hypervariable regions contribute to the formation of the cavity; these are the L1, L3, H1, H2, and H3. The portion homologous to the fourth hypervariable region (residues 82–89), reported in human heavy chains (20), occurs predominantly in the bend B78 at the COOH-terminal end of the variable region and is quite removed from the cavity. L2 likewise does not form a part of the walls of the cavity, being screened from it by the large loop containing L1. The insertions in L1, H2, and H3 cause these hypervariable loops to extend farther out, thus increasing the cavity depth.

The phosphorylcholine occupies only a small part of the cavity and is bound asymmetrically (Fig. 3), being closer to the H chain than to the L chain. The choline moiety is bound in the interior of the cavity with the phosphate group more towards the exterior. The phosphate group appears to be exclusively bound by the H chain; in particular, specific interactions are formed between the phosphate and Tyr 33(H) and Arg 52(H). The hydroxyl group of Tyr 33(H) is apparently hydrogen bonded to one of the oxygens of the phosphate, as is one of the amino groups of the arginine side chain. Moreover, the close proximity of the positively charged guanidinium group of Arg 52(H) to the negatively charged phosphate should produce a large favorable electrostatic interaction. There is also in the immediate vicinity of the phosphate another positive group, that of Lys 54(H), also of the heavy chain which could help neutralize the negative charge of this portion of the hapten. The choline group appears to interact with both the L and H chains. The acidic side chain of Glu 35(H) is about 5 Å away from the positively charged nitrogen of the choline. In addition, there are van der Waals interactions between the choline and main chain atoms

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