Bactericidal Antibody Recognition of Meningococcal PorA by Induced Fit

COMPARISON OF LIGANDED AND UNLIGANDED FAB STRUCTURES*

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MN12H2 is a bactericidal antibody directed against outer membrane protein PorA epitope P1.16 of Neisseria meningitidis. Binding of MN12H2 to PorA at the meningococcal surface activates the classical complement pathway resulting in bacterial lysis. We have determined the crystal structure of the unliganded MN12H2 Fab fragment in two different crystal forms and compared it with the structure of the Fab in complex with a P1.16-derived peptide. The unliganded FabS have elbow bend angles of 155° and 159°, whereas the liganded Fab has a more closed elbow bend of 143°. Substantial differences in quaternary and tertiary structure of the antigen binding site are observed between the unliganded and liganded MN12H2 Fab structures that can be attributed to peptide binding. The variable light and heavy chain interface of the liganded Fab is twisted by a 5° rotation along an axis approximately perpendicular to the plane of the interface. Hypervariable loops H1, H2, and framework loop FR-H3 follow this rotation. The hypervariable loop H3 undergoes conformational changes but remains closely linked to hypervariable loop L1. In contrast with the binding site expansion seen in other Fab-peptide structures, the MN12H2 binding site is narrowed upon peptide binding due to the formation of a “false floor” mediated by arginine residue 101 of the light chain. These results indicate that PorA epitope P1.16 of N. meningitidis is recognized by the complement-activating antibody MN12H2 through induced fit, allowing the formation of a highly complementary immune complex.

Antibody-antigen recognition is considered one of the most specific intermolecular interactions in the immune system. In addition to their antigen binding specificity, antibodies display a variety of secondary biological activities that are critical for host defense. These include virus neutralization, complement activation, opsonization, and signal transduction. Structural aspects of these antibody-associated effector functions have been described for virus-neutralizing antibodies (1–4). The atomic details of the complexes between the Fab fragments of anti-viral antibodies and peptides derived from viral epitopes show common structural features: 1) binding of the peptides induces major rearrangements of the antibody variable domains, and 2) the epitope-peptides bind in tight turn conformations. For a bacterial epitope, we have recently found that it was bound in a similar manner by complement-activating antibody MN12H2, directed against outer membrane protein PorA of Neisseria meningitidis (5).

PorA is a cation-selective transmembrane protein of 44 kDa that forms trimeric pores in the meningococcal outer membrane. According to a topology model, based on known porin structures, the protein is thought to span the membrane in a 16-strand β-barrel conformation (6). The model predicts eight extended extracellular loops. Two of these surface exposed loops (loops 1 and 4) are highly immunogenic and evoke antibodies that induce complement mediated bacterial killing. MN12H2 is a bactericidal antibody that is elicited against loop 4 of PorA. The recognition of loop 4 epitope P1.16 by MN12H2 is used as a model for studying the molecular and structural details of bactericidal antibody recognition of PorA.

The first three-dimensional details of the bactericidal recognition of PorA epitope P1.16 were unveiled in the crystal structure of the MN12H2 Fab fragment in complex with a peptide derived from PorA residues 180–187 (5). The fluorescein-labeled peptide was found in a type I β-turn conformation in the antigen binding cavity. The structure revealed several hydrophobic and electrostatic interactions between both binding partners, including a salt bridge between aspartate 182P of PorA and MN12H2 light chain residue histidine 31L. With the results from a thermodynamic study, this salt bridge was identified as the key interaction explaining the increased incidence of meningitis in United Kingdom in the early 1980s, caused by a D182N mutant strain of N. meningitidis (7, 8).

The capacity of meningococcal PorA to evoke antibodies that induce complement-mediated bacterial killing has incited study to use this protein as a target in vaccine development. In clinical vaccination trials with outer membrane vesicles, it was shown that PorA was critical for the induction of bactericidal antibodies in humans (9). It was also shown that the presence of these antibodies correlate with protection against meningococcal disease (10). The protective activity of the immune complex between an antigen and a bactericidal antibody depends on its ability to cross-link complement factor C1q. With the binding of C1q, the classical complement pathway is activated,

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) U90442 (MN12H2 variable light chain) and U60443 (MN12H2 variable heavy chain).

The atomic coordinates and structure factors (codes 1MPA and 2MPA) have been deposited in the Protein Data Bank, Brookhaven National Laboratory, Upton, NY.

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1 Amino acid residues of the MN12H2 heavy chain and light chain are indicated by H and L, respectively. The peptide residues are indicated by P. Throughout the text, a strict sequential numbering system is used.
which leads to the formation of a multiprotein membrane-attack complex, causing bacterial membrane rupture.

Here, we present the three-dimensional structure of the unliganded MN12H2 Fab fragment at 2.5 Å resolution based on two different crystal forms and compare it with the Fab-P1.16 peptide complex. With both structures available, we examine possible mechanisms by which quaternary and tertiary changes following antigen binding may activate the classical complement pathway.

**EXPERIMENTAL PROCEDURES**

**Preparation of MN12H2 Fab Fragments**—The murine monoclonal antibody MN12H2 was purified from hybridoma cell culture supernatant as described previously (11). The Fab fragment was obtained by papain digestion with papain-agarose beads (Sigma) at 37 °C for 4–16 h. The digestion buffer consisted of 10 mM Tris-HCl (Fluka, Buchs, Germany), pH 7.4, 1 mM EDTA, 0.02% w/v NaN3, and 1 mM dithioerythritol. After digestion at least four prominent Fab isoforms could be identified by isoelectric focusing (IEF) (Pharmacia LKB, Uppsala, Sweden) with approximate pI values of 8.45, 8.65, 9.1, and 9.3. The IEF pattern of MN12H2 Fab-peptide crystals showed that the crystallized Fab fragment mainly consisted of the pI 8.65 isoform. Isolation of this isoform was performed by anion exchange chromatography using a Q-Sepharose column (Amersham Pharmacia Biotech). The Fab fraction of the papain digest was loaded to the column using 20 mM 3-(cyclohexylamino)-1-propane sulfonic acid (CAPS) (Fluka), pH 9.8, 0.02% w/v NaN3 as a binding buffer. The different Fab isoforms were eluted with a 0–15% NaCl gradient. The recovered isoforms were analyzed by SDS-polyacrylamide gel electrophoresis, IEF, electron-spray mass spectrometry, and dynamic light scattering using a DynaPro-801 dynamic light scattering instrument (Protein Solutions Ltd., High Wycombe, Buckinghamshire, United Kingdom). Specific binding of the purified pI 8.65 Fab isoform to the P1.16 epitope was determined by means of fluorescence polarization experiments with a synthetic epitope peptide, as described earlier (8).

**Crystallization and Data Collection**—Preliminary crystallization conditions of the unliganded MN12H2 Fab were identified with a set of screening solutions using concentrations of 15–45% v/v polyethylene glycol 3000 (Fluka) as precipitating agents in combination with low molar concentrations of CaCl2, MgCl2, and CdCl2. The screening solutions were collected from a Nonius FR570 rotating anode (Nonius, Delft, The Netherlands) operated at 45 mA and 95 mA. Diffraction data were auto-indexed and processed with DENZO and SCALEPACK (12).

Data were collected from two different Fab crystal forms obtained under slightly different conditions (27 and 20% v/v MPD). Crystals grown at 27% MPD were C-centered orthorhombic C222, with cell dimensions a = 86.0, b = 114.9, c = 153.1 Å. Diffraction to about 3.2 Å resolution was collected to 100% completeness from a single crystal of approximate size 0.2 × 0.2 × 0.15 mm3. The asymmetric unit contains one Fab molecule with a Matthews coefficient (Vm) (13) of 3.8 Å3/Da and a solvent content of 68%. The 20% MPD crystals belonged to the C-centered monoclinic space group C2, with unit cell dimensions a = 114.6, b = 85.9, c = 87.1 Å and β = 122.7°. A complete data set was collected from a single crystal with dimensions of 0.2 × 0.2 × 0.15 mm3 diffracting to 2.5 Å resolution. The monoclinic crystal form also contains one Fab in the asymmetric unit, Vm is 3.6 Å3/Da, with a solvent content of 66%. Crystallization and data collection details are summarized in Table I.

**Molecular Replacement and Refinement**—The structures of the unliganded MN12H2 Fab were solved by molecular replacement using the MN12H2 Fab of Protein Data Bank entry 1MPA as a search model. The rotation function, the Patterson correlation function optimizing the mutual orientation of the four Fab domains (VL, VH, CL, and CH1), the translation function, and structure refinement were performed with the program Crystallography and NMR System (14–17). Refinement used the maximum likelihood target with all experimental amplitudes except for a randomly selected test set of 10% that was used for cross-validation a weighting. The automated refinement procedure consisted of conjugate minimization followed by torsion angle molecular dynamics in combination with simulated annealing. Refinement was preceded by calculation of a bulk-solvent model, estimate a, and weight values. Rouns of refinement were followed by rebuilding of the models in O (18) using a weighted maps. Finally, the unliganded Fab models were subjected to restrained B-factor refinement.

The structure of the MN12H2 Fab in complex with the P1.16-derived fluorescein-conjugated peptide was also subjected to the maximum likelihood target and torsion angle dynamics refinement method.

**RESULTS**

**Structure Determination of the Unliganded Fab**—The structure of the unliganded Fab was solved for two different crystal forms. The monoclinic crystal form (space group C2) diffracted to 2.5 Å resolution, whereas the orthorhombic crystal form (space group C222,) diffracted to a resolution of 3.2 Å. Crystalization and data collection statistics are given in Table I.

Structures were refined to an R factor of 23% and an Rfree of

![Image](image.png)
27% for the monoclinic crystal form and to an R factor of 25% and an Rfree of 30% for the orthorhombic crystal form. Geometric parameters evaluated with PROCHECK (19) and WHAT IF (20) show acceptable values for both crystal structures. Refinement and model statistics are given in Table II. Almost 90% of the residues of the monoclinic crystal form of the unliganded Fab had main chain torsion angles that fell within the energetically most favored regions of the Ramachandran plot (21), whereas none were found in the disallowed regions (Fig. 1A).

### Table II

| Refinement/statistics                        | Unliganded Fab, orthorhombic | Unliganded Fab, monoclinic |
|---------------------------------------------|------------------------------|----------------------------|
| Resolution (Å)                             | 20–3.2                       | 20–2.5                     |
| R (%)                                       | 25.3                         | 23.1                       |
| Rfree (%)                                   | 29.8                         | 27.0                       |
| Cadmium ions (no.)                         | 4                            | 3                          |
| Water molecules (no.)                       | 5                            | 196                        |
| Root mean square deviations from ideality   |                              |                            |
| Bonds (Å)                                   | 0.014                        | 0.008                      |
| Angles (°)                                  | 1.69                         | 1.62                       |
| Dihedrals (°)                               | 25.7                         | 26.3                       |
| Improper dihedrals (°)                      | 0.77                         | 0.79                       |
| Average B-factors (protein) (Å²)            | ND*                          | 23                         |
| Cross-validated a coordinate error (Å)      | 0.71                         | 0.34                       |

*ND, not determined. B-factors were taken from the monoclinic unliganded MN12H2 Fab.

As in the MN12H2 Fab-peptide complex (5), strong electron density peaks were seen in both unliganded Fab crystal forms. Because cadmium ions were essential for crystallization and because of the vicinity of putative cadmium binding residues, the positions of these 5σ electron density peaks are very likely to be occupied by cadmium ions. As in the 1MPA structure, a putative cadmium binding site was found near the Nδ1 of His-98L. In the monoclinic unliganded Fab structure, this cadmium is coordinated by two water molecules, as in the 1MPA structure, and by the carboxylate oxygens of Glu-218L at the N terminus of a symmetry related molecule. A second cadmium...
ion interacts with the Nε2 of His-172H, the Nε2 of Asn-143L, and a water molecule. The third cadmium was coordinated by the carboxylate oxygens of Glu-190L, the Nε2 nitrogens of His-194L, and a water molecule and via crystal packing interactions with the carboxylate moiety of a symmetry-related Asp-181H. In the orthorhombic crystal form, an additional cadmium ion was observed that occupied a special position situated on a 2-fold rotation axis parallel to b. The ion was found to be coordinated by the carboxylate oxygens of four glutamate residues, including Glu-84L, Glu-86L, and their symmetry-related residues. However, some of the densities assigned to coordinating water molecules may actually be occupied by chloride ions.

**Maximum Likelihood Refinement of the 1MPA Structure**—Because the structures of the free and complexed MN12H2 Fab were refined using different techniques, for good comparison of both models, the Fab-peptide structure (Protein Data Bank entry 1MPA) was subjected to refinement using the maximum likelihood target and torsion angle dynamics refinement method. The 1MPA structure of the MN12H2 Fab-P1.16 peptide complex was refined using the least squares target function. The 2MPA structure resulted from refinement of 1MPA using the maximum likelihood target and torsion angle dynamics refinement method.

**Comparison of the Free and Complexed MN12H2 Fab Structures**—We observed elbow bend angles of 155° and 159° for the unliganded Fab in the monoclinic crystal form and the orthorhombic crystal form, respectively. For the liganded MN12H2 Fab, a more closed elbow bend was found with an angle of 143° between the pseudo-2-fold rotation axis of the V and C superdomains (Table III).

**Table III**

|                     | Unliganded Fab, orthorhombic | Unliganded Fab, monoclinic | Liganded Fab |
|---------------------|------------------------------|----------------------------|--------------|
| Pseudodyad angles   |                              |                            |              |
| Vι-Vι dimer         | 175°                         | 176°                       | 175°         |
| C1-C1 dimer         | 168°                         | 168°                       | 168°         |
| Elbow bend angles   |                              |                            |              |
| V-C domains         | 159°                         | 155°                       | 143°         |

**Table IV**

|                     | Fab-peptide complex, 1MPA (6) | Fab-peptide complex, 2MPA |
|---------------------|-----------------------------|---------------------------|
| Resolution          | 8-2.6                       | 20-2.6                    |
| R (%)               | 19.4                        | 20.2                      |
| R<sub>free</sub> (%) | 30.9                        | 26.4                      |
| Cadmium ions (no.)  | 1                           | 1                         |
| Water molecules (no.) | 17                        | 52                        |
| Root mean square deviations from ideality |                |
| Bonds (Å)           | 0.011                       | 0.008                     |
| Angles (°)          | 1.89                        | 1.53                      |
| Dihedrals (°)       | 27.3                        | 25.5                      |
| Improper dihedrals (°) | 1.51                     | 0.77                      |
| Average B-factors (protein) (Å²) | 28 | 44               |
| Cross-validated σa coordinate error (Å) | 0.45 | 0.48 |

A 5° rotation was observed between the variable domains of the unliganded Fabs and the Fab-peptide complex, along an axis approximately perpendicular to the Vι-Vι interface. As illustrated in Figs. 2 and 3A, the largest coordinate differences resulting from this rotation were found at the tips of the hypervariable loops (C<sub>a</sub>-coordinate differences up to 3.6 Å for hypervariable loop H1 and up to 3.3 Å for H2). The H3 loop does not follow this domain rotation, and its backbone is relatively kept in position with only minor C<sub>a</sub>-coordinate differences from 0.2 Å for Asp-105H and up to 1.6 Å for Ala-104H. As depicted in Fig. 3B, the H3 loop is fixed to loop L1 by a tandem of Tyr-Asp hydrogen bonds. The side chain hydroxyls of tyrosine residues 37L and 41L bind to the carboxylate oxygens of aspartate residues 102H and 109H of loop H3. These Vι<sub>1</sub>-Vι<sub>1</sub> interface interactions are further tightened by a hydrogen bond between the Nε2 of hypervariable loop L2 residue Lys-55L and the carbonyl oxygen of Phe-101H (loop H3).

Major side chain displacements between the unliganded Fabs and the Fab-peptide complex were observed in loops L3 and H3. In both unliganded Fabs, arginine residue 101L protrudes from the floor of the antigen binding site (Fig. 3C), thereby creating a positively charged bulge. This is also illustrated in the molecular surface of the unliganded antigen binding site in Fig. 4A, colored for electrostatic potential. In contrast, Arg-101L spans the binding pocket in the Fab-peptide complex, and its guanidinium group forms hydrogen bridges with residues Ser-97H and Tyr-41L. In the newly refined liganded Fab structure, additional interactions are seen with Arg-101L: a water-mediated hydrogen-bonding interaction with the hydroxyl group of Ser-94 (hypervariable loop L3) and a hydrogen bond with Nε2 of His-35H (hypervariable loop H2). The largest atomic displacements between the free and the complexed Fab were observed in the H3 loop. As illustrated in Figs. 3B and 4A, a dramatic displacement was seen for Tyr-103H with a 9-Å difference of the hydroxyl oxygens between both structures. The hydroxyl oxygen of Tyr-100H, pointing toward the binding site in the unliganded Fab, is moved away 3.4 Å from the cavity in the Fab-peptide complex and points upward.
Crystal Contacts—Between both free Fab crystal forms and the Fab-peptide complex, the hypervariable loops play different roles in crystal packing interactions. In the monoclinic as well as the orthorhombic unliganded Fab L3 loop, residue His-98L is involved in head-to-tail symmetry interactions with the C terminus of the light chain, mediated by a cadmium ion (defining the variable domain as head and the constant domain as tail). In the monoclinic crystal form, 70% of all hypervariable loop crystal contacts involve additional head-to-tail van der Waals interactions between the tips of loops L1 and L2 and the interchain disulfide region. Also, several salt links with the C-terminal region of the CH1 domain of a symmetry-related molecule are observed. The interactions between loop L1 and the interchain disulfide residues Gly-137H and Thr-140H in the monoclinic unliganded Fab result in unexpectedly well-defined electron density for this archetypically disordered region. In the orthorhombic Fab, no interactions are observed between the three light chain hypervariable loops and the interchain disulfide region. In both unliganded Fab structures, the H3 loop is packed head-to-head with the binding site of a symmetry-related molecule.

In the complexed Fab, crystal contacts mainly involve head-to-head packing between the apices of hypervariable loop L1 and loops H1 and H3 of a symmetry-related molecule. The fluorescein label of the peptide accounts for 11 additional head-to-tail contacts with the C terminus of the CH1 domain. A single interaction is observed between loop L1 and the interchain disulfide region. In contrast with the free Fab crystal forms, the liganded Fab displays crystal contacts with elbow bend residues 112L, 113L, and 114L that are packed against the heavy chain of a symmetry-related molecule.

DISCUSSION

Comparison of two crystal forms of the unliganded MN12H2 Fab with the liganded structure of the epitope-peptide Fab complex reveals significant conformational changes. The surface representations of the binding sites of both structures in Fig. 4 indicate the quaternary and tertiary changes of the Fab upon peptide binding. The antigen binding site of MN12H2 may be thought of as a left-handed baseball glove, with hypervariable loops L1 and L3 forming the thumb, and the VH domain (with loops H3, H2, and H1) as the fingers. As in the baseball glove, the thumb (loop L1) and the forefinger (loop H3) remain connected upon peptide binding through a “two-bar” web formed by a tandem of tyrosine-aspartate interactions, whereas the other fingers (loops H2, framework region-H3, and H1) follow the shape of the peptide (see also Fig. 2). In the unliganded Fab, arginine residue 101L forms a positively charged bulge in the binding cavity. Upon peptide binding, this bulge is depressed, and its charge is neutralized due to the
formation of a false floor that narrows the cavity and connects four hypervariable loops (L1, L3, H1, and H3). The side chain rearrangements in the H3 loop represented by tyrosines 100H and 103H are necessary to clear the binding site to further accommodate the peptide binding. When the P1.16 peptide is positioned in the binding site of the unliganded Fab, the side chain of these residues sterically blocks the binding of the peptide.

The sterical hindrance of hypervariable loop H1 residues that is encountered by the peptide is overcome by the observed 5° twist of the VL-VH interface in the liganded Fab. This rotation brings H1 residue Tyr-33H in position to form a hydrophobic stack with peptide residue Thr-183P. The VL-VH twist also decreases the distance between the Cα atoms of hypervariable loops L1 (Arg-101L) and H3 (Ser-97H) from 13 to 11.5 Å, thereby facilitating the formation of a Arg-101L-mediated bidentate hydrogen bond with the side chain hydroxyl oxygen of Ser-97H and the hydroxyl oxygen of Tyr-41L. The hydrogen bond between the NH2 group of Arg-101L and ND2 of His-35H (hypervariable loop H2) induces an almost 90° rotation of the 35H imidazolium ring bringing the NE2 nitrogen in position to fix the peptide backbone at the newly formed cavity floor. The formation of this false floor, directed by Arg-101L, stabilizes the topography of the complexed VL-VH dimer by locking four hypervariable loops (L1, L3, H1, and H3) into a new conformation. A thermodynamic study on the interaction with MN12H2 and the P1.16 peptide revealed that these interactions, together with the release of structured water from the binding pocket and the newly formed interactions between Fab and peptide, favor the interaction by 100 kJ mol⁻¹ (8). The largely offsets the 50 kJ mol⁻¹ cost in free energy needed to induce the structural changes in both binding partners (as determined by the analysis of Sturtevant (22)).

With the exposed guanidinium moiety of Arg-101L in the cavity surrounded by basic residues His-31L and Arg-59L, an overall positively charged binding site is formed in the unliganded Fab, as illustrated in Fig. 4A. Although Arg-101L does not contribute directly to peptide binding, it could explain the medium-affinity cross-reactive binding mode of MN12H2 for negatively charged self-antigens such as single stranded DNA and cardiolipin.3 The appearance of basic residues at these sites within the light chain hypervariable loops and especially at the junction between Vκ and Jκ genes (101L) fits the general pattern of DNA-binding antibodies described by Radic and Weigert (23). Whether the binding of these autoantigens adopt different complexed structures remains to be determined.

Relative disposition of Vκ-VH domains has been observed before in Fab structures upon binding of a peptide (1, 24, 25). It illustrates the intrinsic flexibility of antibodies in adapting to the shape of an antigen. The largest VL-VH rotations have been observed for anti-DNA Fab BV0–401 (7.5°) (26) and for anti-HIV Fab 50.1 upon complexation with a V3 loop peptide (16°) (25). Unlike MN12H2, these Fab structures show a large decrease in the number of VL-VH interface contacts upon complexation with their antigen. The loss of self-contacts is mainly ascribed to rearrangements of the H3 loop, which moves out of the binding site with root mean square deviations in backbone atoms up to 5 Å. In contrast with this binding site expansion, the MN12H2 Fab shows a closer association of both domains upon peptide binding by forming a false floor. The 5° rotation of the H1 and H2 loop narrows the cavity even more and holds the peptide in a tight lock. Because the backbone of the H3 loop remains fixed, massive side-chain rearrangements of tyrosines 100H and 103H are essential for the binding site to adapt to the shape of the peptide.

All of the structural changes mentioned in this section appear to be necessary for peptide binding. Because both monoclinic and orthorhombic crystal forms of the unliganded Fab display similar features in a different crystal packing environment, the conformational differences with the liganded Fab are believed to be peptide-induced. No conformational changes of fluorescein binding residue Arg-59H have been detected between the unliganded Fabs and 1MPA, suggesting no or only

3 J. M. H. van den Elsen, unpublished results.
minor effects of the fluorescein molecule on the shaping of the binding site.

Conformational changes in the elbow bend angle and the distal extremities of the constant region, such as the interchain disulfide region, are believed to be induced by the general flexibility of these regions and by their different crystal packing interactions. The elbow bend regions at the junction between the variable and constant domains are very flexible and there have been differences found in the elbow bend angle between unliganded structures as well as liganded structures of the same Fab (27). Stanfield et al. (25) even reported a 13° difference in elbow bend angle between two complexes of 50.1 Fab molecules found in the same asymmetric unit, also illustrating this general flexibility.

There is no evidence that the peptide-induced conformational changes, other than adjusting fit and complementarity to the antigen, provide a signal that is transmitted to the constant domains triggering secondary biological activities. The accessibility and the tight-turn recognition motif of immunogenic domains triggering secondary biological activities. The accessibility in elbow bend regions at the junction between two complexes of 50.1 Fab molecules found in the same asymmetric unit, also illustrating this general flexibility.

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