Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Use of streptococcal protein G in obtaining crystals of an antibody Fab fragment in complex with a meningococcal antigen

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Received 25 April 1998
Accepted 28 July 1998

Crystals have been obtained of an antibody Fab fragment grown in the presence of a single domain from streptococcal protein G and a ten amino-acid peptide corresponding to the P1.7 serosubtype antigen from the human pathogen *Neisseria meningitidis*. Crystal trials using the Fab fragment and peptide antigen alone were unsuccessful, but the inclusion of a protein G domain provided an additional variable that generated suitable crystals. Crystals are in space group $P2_1$ with unit-cell parameters a = 43.60, b = 63.42, c = 89.63 Å, β = 98.58° and a data set has been collected to 2.9 Å resolution using synchrotron radiation. The inclusion of protein G is likely to be of general utility for the crystallization of Fab–antigen complexes.

1. Introduction

The bacterium *N. meningitidis* is the causative agent of meningococcal meningitis, and the development of novel vaccines providing protection against Group B meningococcal infection is a priority. Vaccines currently under development include the porin protein PorA, which is a principal protein component of the outer membrane of most meningococcal isolates. This protein has been postulated to adopt a 16-stranded transmembrane β-barrel structure with eight surface-exposed loops (I–VIII) on the basis of sequence homology with porin proteins from *Escherichia coli* (Cowan *et al.*, 1992). The amino-acid sequences of the longest of the putative surface loops, I and IV, are highly variable among meningococcal isolates, and these loops are thought to protrude above the membrane surface and interact with the host. This variation is the basis of the serosubtyping scheme for meningococci and a number of monoclonal antibodies are available which react with different serosubtypes (Frasch *et al.*, 1985). The serosubtyping of a given meningococcal isolate is based upon the recognition of distinct epitopes present in loops I and IV of PorA by independent antibodies. Therefore, each isolate has two subtype designations which are conventionally written after the prefix 'Pl.' and separated by a comma in the order loop I, loop IV (*e.g.* P1.7,10). Comparative sequence analyses have shown that the variability of both loop I and loop IV comprises (i) different families of peptide sequence, which are designated by a number (*e.g.* P1.7), and (ii) minor variants within these families, which are designated by an additional letter (*e.g.* P1.7, Pl.7a, *etc*). Most serosubtyping antibodies recognize linear epitopes, which can be identified by peptide scanning (McGuinness *et al.*, 1993). As the loop sequences are larger than the minimal epitope necessary for antibody recognition, the minor variants may or may not react with a given serosubtyping antibody. In the case of the P1.7 serosubtype, four sequence variants have been identified (P1.7, Pl.7a, Pl.7b and Pl.7c), all of which cross-react with the same monoclonal antibody. In view of the importance of the recognition of PorA by antibodies, both in the immune response to prospective vaccines and in the serosubtyping of meningococci, we have undertaken an X-ray crystallography study of the binding of this antibody to the P1.7 epitope.

Structural studies on antibody–antigen interactions have, in many cases, been plagued by difficulties in obtaining suitable crystals of the appropriate Fab-fragment–peptide-antigen complex. The bacterial immunoglobulin-binding protein, protein G from *Streptococcus*, offers an alternative approach to this problem. The IgG-binding domains within the protein G gene have been identified as three highly homologous sequences which retain high affinity for either the Fab or Fc portion of IgG when expressed individually (Guss *et al.*, 1986). Crystal and NMR structures of these domains have demonstrated that the secondary structure is made up of a central α-helix packed against a four-stranded β-sheet (Derrick & Wigley, 1992; Lian *et al.*, 1992). Recognition of the Fab portion of IgG is almost exclusively *via* the C_H1 domain, and does not interfere with antigen binding (Derrick & Wigley, 1992). In this work, we have shown that one of these protein G domains can be included as an additional variable in crystallization trials for Fab fragments and describe the diffraction properties of the new crystal form obtained. This is the first report of the crystallization of

an Fab–protein-G–peptide-antigen ternary complex.

2. Experimental and results

The hybridoma MN14C11.6 (Abdillahi & Poolman, 1988) secreting the anti-P1.7 antibody was used for the production of ascitic fluid in pristane-treated Balb/C mice. The antibody (IgG2κ) was purified from ascites fluid by chromatographic separation on a Sepharose column of immobilized protein G, as described previously (Derrick *et al.*, 1992). Purified antibody from the column was concentrated to a final concentration of 2.7 mg ml^{-1} and dialysed extensively against 50 m*M* sodium acetate/acetic acid (pH 5.5). Proteolytic cleavage of the antibody was then initiated by addition of dithiothreitol, EDTA and papain to final concentrations of 50 m*M*, 1 m*M* and 8 µg ml^{-1}, respectively; proteolysis was allowed to proceed for 5.5 h at 310 K. The solution was then dialysed for 12 h at 277 K *versus* 1 l of 50 m*M* sodium acetate/acetic acid (pH 4.5). Fab fragments were separated from Fc fragments and undigested IgG by cation-exchange chromatography on a Mono-S 10/10 FPLC column (Pharmacia), equilibrated in 50 m*M* sodium acetate/acetic acid (pH 4.5, buffer *A*). Fab fragments were eluted from the column with a linear gradient from buffer *A* to buffer *A* plus 1 *M* NaCl (pH 7.5), with the Fab fragments eluting in one major peak at approximately 130 m*M* NaCl. Peak fractions containing the Fab fragment were transferred into the crystallization buffer by concentration over a YM10 Amicon membrane, dialysed against 1 l of 20 m*M* sodium acetate/acetic acid (pH 5.5) and subsequently concentrated to a final protein concentration of 20 mg ml^{-1} in a Centricon-10 concentrator (Amicon). The protein concentration of the Fab fragment was estimated assuming an absorbance coefficient of 70 mM^{-1} cm^{-1} at 280 nm, calculated from the amino-acid sequences of the heavy and light chains (Gill & von Hippel, 1989).

For the protein G, a single-site mutant (E24K) of protein G domain II was used for the crystal trial; protein G domain II is a 64-residue protein previously described by Lian *et al.* (1992). Protein G domain II differs by only four amino-acid residues from domain III, the protein G domain that was used by Derrick & Wigley (1992) to study the recognition of a mouse Fab fragment by solution of the crystal structure of a protein G–Fab complex. The E24K mutant of protein G domain II was used because this mutation accounts for the threefold higher binding affinity of domain III for Fab compared to domain II (J. Derrick, unpublished results). The E24K mutation was introduced into the coding sequence for protein G domain II in mp18 (described in Lian *et al.*, 1992) using the deoxyuridine-selection protocol described by Künkel *et al.* (1987). Purification of the mutant E24K protein G domain II was conducted as described by Lian *et al.* (1992) for the wild-type protein.

Previous epitope-mapping studies have shown that the minimal amino-acid sequence recognized by this antibody is centred on the tripeptide SGQ within the P1.7 sequence AQAANGGASGQ-VKVTKVTKA (McGuinness *et al.*, 1990). Several different peptides corresponding to the P1.7 antigen sequence were used for crystal trials, but the best results were obtained using the decamer ANGGASGQVK. Crystal trials were conducted using the hanging-drop method and a random-factorial screening method was initially used to identify suitable crystallization conditions, as described by Jancarik & Kim (1991). Initial crystal trials with the decamer peptide and Fab fragments failed to produce satisfactory crystals, although several different ratios of Fab to peptide were used. Successful crystallization conditions were identified, however, when protein G domain II containing the E24K mutation was included with the peptide antigen and Fab fragment. The Fab fragment, protein G and peptide were premixed immediately prior to crystallization to give molar proportions of 1:5:8 (Fab:protein G:peptide) at a total protein concentration of 21 mg ml^{-1}. The concentrations of the constituents were 11 mg ml^{-1} Fab, 8.1 mg ml^{-1} protein G and 2.2 mg ml^{-1} peptide. Crystallization was carried out by the hanging-drop method; the reservoir contained 50 m*M* sodium acetate/acetic acid (pH 5.0) and 24%(*w*/*v*) PEG 6000 and the droplet was formed by mixing 4 µl of the Fab/protein G/peptide solution with 4 µl of the reservoir. Thin needle-shaped crystals formed within 5 d at 293 K to a maximum size of 50 × 50 × 300 µm. Better formed and larger crystals could be grown by macro-seeding these initial crystals into a Fab/protein G/peptide solution in 14% PEG 6000 and 50 m*M* sodium acetate/acetic acid (pH 5.0). Formation of the crystals was dependent on the inclusion of the decamer peptide antigen; no crystals were obtained if it was omitted.

Data collection was carried out on station 9.5 at the SRS, Daresbury, using radiation of wavelength 0.92 Å. Immediately before data collection, the crystals were harvested into a cryoprotectant solution of 22.5% PEG 6000, 10% glycerol and 50 m*M* sodium acetate/acetic acid (pH 5.0) and soaked for 5 min; the crystals were then frozen by rapid transfer to a nitrogen-gas stream at 100 K. A data set was collected to 2.9 Å resolution from a single crystal (50 × 100 × 200 µm) using the oscillation method (Arndt & Wonacott, 1977), with an oscillation range of 2°, a crystal-to-film distance of 450 mm and an exposure time of 2 min per image. The data were processed and scaled using the programs *DENZO* and *SCALEPACK* (Otwinowski, 1986) to give an $R_{\rm sym}$ of 2.7% for a total data set of 52796 reflections, with 9953 unique reflections (89.1% complete to 2.9 Å). The space group was determined to be $P2_1$, with $a = 43.60$, $b = 63.42$, $c = 89.63$ Å, $\beta = 98.58°$. Assuming one Fab–protein-G–peptide molecule in the asymmetric unit, the packing volume V_m was determined to be 2.76 Å^3 Da^{-1} (Matthews, 1968). Work is currently in progress to solve the structure by molecular replacement using the Fab–protein G complex as a starting model.

We are grateful to Liz Duke and Rob Kehoe for assistance with data collection on station 9.5, to Dr Jan Poolman for the supply of the P1.7 hybridoma and to Dr Mark Achtman for the P1.7 peptide. We also thank Janet Suker for technical assistance. JPD is a Fellow of the Lister Institute of Preventive Medicine and MCJM is a Wellcome Trust Senior Fellow in Basic Biodiversity Research.

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