CRYSTALLIZATION NOTES

Crystallization and Preliminary X-ray Analysis of the Complex between a Mouse Fab Fragment and a Single IgG-Binding Domain from Streptococcal Protein G

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The Fab fragment of a mouse immunoglobulin G1, complexed with a single IgG-binding domain from streptococcal protein G, has been crystallized in a form suitable for analysis by X-ray diffraction. The needle-shaped crystals were grown from polyethylene glycol 4000 using vapour diffusion methods and diffract to 2.3 Å resolution. The space group is P2₁2₁2₁ (a = 64.5 Å, b = 70.5 Å and c = 120.1 Å), with one Fab–protein G domain complex in the asymmetric unit. Solution of the three-dimensional structure of the complex will permit a detailed analysis of the molecular interactions between protein G and the Fab portion of IgG.

Keywords: crystals; Fab; antibody; protein G; Streptococcus

Protein G is a member of a diverse group of cell-surface proteins from Gram-positive bacteria that have high affinity for immunoglobulins. These proteins may play a role in the ability of a pathogen to adsorb serum proteins and thus evade the host immune defences. In addition to their contribution to virulence, bacterial immunoglobulin-binding proteins have also attracted considerable interest due to their many applications in antibody labelling and purification (Kronvall, 1990). An understanding of the molecular basis of the interaction between protein G and IgG† is therefore important in the development of protein G molecules with novel specificities by protein engineering methods.

At present, the two best-understood bacterial immunoglobulin-binding proteins from a structural viewpoint are protein A (from Staphylococcus aureus) and protein G (from group C and group G streptococci). Both proteins are known to bind to the Fe portion of IgG with high affinity and have therefore found widespread application in the purification of antibodies. Protein A and protein G have also been reported to bind to Fab fragments of IgG, although this behaviour is rather less well characterized (Myhre, 1990). The interactions of protein G and protein A are presumably with the conserved regions of the Fab molecule and form an interesting contrast with Fab–antigen complexes.

The IgG-binding domain from protein A is made up of 58 residues and forms an α-helical structure in solution. Detailed information on the interaction of protein A with the Fe portion of IgG has been obtained by the solution of the X-ray crystal structure of human Fe in complex with a single IgG-binding domain (Deisenhofer, 1981). This work demonstrated that two α-helices from protein A make extensive side-chain–side-chain contacts with residues at the interface between the C₃₅₂ and C₃₃ domains in Fe. Studies of a single IgG-binding domain from protein G by ¹H nuclear magnetic resonance have demonstrated that the secondary structure is made up of one α-helix packed onto a four-stranded β-sheet, a fundamentally different arrangement from protein A (Lian et al., 1991). To determine the mechanism of interaction of protein G with the Fab portion of IgG, we set out to obtain crystals of a Fab-protein G complex.

Murine IgG1 (kappa) was purified from mouse

†Abbreviations used: IgG, immunoglobulin G; f.p.l.c., fast protein liquid chromatography.
ascites fluid, derived from MOPC 21 cells, by fractionation on a protein G-Sepharose column, using the same method as described for protein A-Sepharose (Harlow & Lane, 1988). Fab fragments were prepared by digestion of IgG1 (2-1 mg/ml) with papain (4-6 μg/ml) in 0-1 M-sodium acetate (pH 5-5), 50 mM-cysteine and 1 mM-EDTA at 37°C. The progress of digestion was monitored by SDS/polyacrylamide gel electrophoresis using a 12.5% gel. After digestion was complete, the products were transferred to buffer A (10 mM-Tris-HCl (pH 7-5)) by f.p.l.c. gel filtration using an HR 16/10 rapid desalting column (Pharmacia). Fab fragments were then purified by fractionation on an f.p.l.c. anion exchange resin (Mono Q 10/10 column; Pharmacia) using a linear gradient from buffer A to buffer A plus 0.25 M-NaCl (total gradient volume = 80 ml). One Fab isoform, which eluted at 40 mM-NaCl, was the dominant product and fractions containing this peak were pooled and lyophilized. (The purity of this product was verified by isoelectric focussing using a Mono P 5/20 f.p.l.c. column, with a pH gradient from 9.3 to 6.0.) The dried product was redissolved in 0.7 ml of water and desalted against 5 mM-Mes/NaOH (pH 5-5) plus 100 mM-NaCl. Recombinant protein G domain III, isolated from Escherichia coli (Lian et al., 1992), was added to a threefold molar excess over Fab fragments and left for two hours at 4°C. The solution was then concentrated to approximately 200 μl in an Amicon Centricon 10 and washed three times with 5 mM-Mes/NaOH (pH 5-5) to generate a stock solution at approximately 24 mg protein/ml total concentration. Crystals were grown using a vapour diffusion method from hanging drops, with 10 μl drops and 2 ml reservoirs. To form the droplet, 5 μl of stock protein solution was mixed with 5 μl of the reservoir, which consisted of 20 mM-Tris-HCl (pH 6-5) plus 8% polyethylene glycol 4000. The hanging drops were transferred to a constant temperature incubator at 20°C and needle-shaped crystals, approximately 1 mm long by 0.13 mm wide, appeared after five days.

Crystals were analysed at the EMBL Outstation in Hamburg. The crystals proved to be orthorhombic, space group P212121, with cell dimensions of \( a = 64.5 \text{ Å}, \quad b = 70.5 \text{ Å}, \quad c = 120.1 \text{ Å} \). \( 1 \text{ Å} = 10^{-10} \text{ m} \). The asymmetric unit apparently contains one protein G-Fab complex \( (V_m = 2.28 \text{ Å}^3) \). (Matthews, 1968). Data were collected to 2.6 Å from a single crystal; these are currently being used in molecular replacement studies.

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References


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