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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 $P2_12_12_1$ ($a = 64{\cdot}5$ Å, $b = 70{\cdot}5$ Å and $c = 120{\cdot}1$ 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 Fc 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 Fc portion of IgG has been obtained by the solution of the X-ray crystal structure of human Fc 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 C_H2 and C_H3 domains in Fc. 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.

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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-bis-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 $P2_12_12_1$, with cell dimensions of $a = 64{\cdot}5$ Å, $b = 70{\cdot}5$ Å, $c = 120{\cdot}1$ Å (1 Å = 10^{-1} nm). The asymmetric unit apparently contains one protein G–Fab complex ($V_m = 2{\cdot}28$ Å^3/Da; 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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