Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

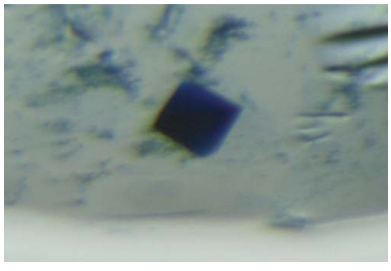
Crystallization and preliminary crystallographic analysis of recombinant immunoglobulin G-binding protein from *Streptococcus suis*

Abdul Hamid Khan,[a] Fuliang Chu,[a] Youjun Feng,[a] Qinagmin Zhang,[a] Jianxun Qi[b] and George Fu Gao[a]*

[a]Center for Molecular Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, People's Republic of China, and [b]Institute of Physics, Chinese Academy of Sciences, Beijing 100080, People's Republic of China

Correspondence e-mail: gaof@im.ac.cn

Received 14 March 2008
Accepted 1 July 2008



Streptococcus suis, an important zoonotic pathogen, expresses immunoglobulin G-binding protein, which is thought to be helpful to the organism in eluding the host defence system. Recombinant IgG-binding protein expressed in *Escherichia coli* has been crystallized using the hanging-drop vapour-diffusion method. The crystals belonged to space group $P2_12_12_1$, with unit-cell parameters $a = 38.98$, $b = 43.94$, $c = 78.17$ Å and one molecule in the asymmetric unit. Diffraction data were collected to 2.60 Å resolution.

1. Introduction

The expression of surface receptors that bind immunoglobulins in a non-immune fashion by a variety of human and animal pathogens is evident from previous work (Balber & Sturtevant, 1986; Bricker *et al.*, 1991; Stannard & Hardie, 1991). An association between the expression of these molecules and the severity of disease has been demonstrated by epidemiological analysis. Establishment of a pathogenic infection involves a complex interaction between bacteria and the host (Miller *et al.*, 1989). To prevent colonization and attachment of bacteria, a variety of specific and nonspecific defence mechanisms are used by the host. To survive in the hostile environment of the host, the pathogen needs a means of circumventing the host defence mechanisms. Depending on the site of infection, pathogenic organisms use different virulence factors to attach and colonize: the host defence mechanism at mucosal surfaces might be expected to be contributed by locally produced immunoglobulin A (IgA) antibodies (Underdown & Schiff, 1986), while the roles of neutrophils and immunoglobulins of the IgG class might be expected to be more predominant in skin infections (Boyle & Borsos, 1983). As mentioned previously, various human and animal pathogens have been shown to carry a surface receptor that is capable of interacting with IgG in a non-immune fashion (Bessen & Fischetti, 1990; Blumenfeld *et al.*, 1990; Ferher *et al.*, 1988; Widders *et al.*, 1989). Studies carried out by Bessen and Fischetti on group A streptococci have also demonstrated an increased ability to bind human IgG in a non-immune fashion using isolates recovered from the skin when compared with those isolated from the nasopharynx (Bessen & Fischetti, 1990). The ability of group A organisms to establish lethal infections in mice after injection into a skin air sac was associated with enhanced expression of IgG-binding proteins. The results of these studies suggest that the ability of bacteria to express IgG-binding activities may contribute to the pathogenicity of group A organisms, in particular for infections of the skin.

2. Materials and methods

2.1. Cloning, expression and purification

The gene encoding IgG-binding protein (IgGBP; 05SSU0229) was amplified and cloned into the vector pET-30a (Novagen). Positive cloning was confirmed by double-enzyme digestion and verified by direct DNA sequencing. Competent *Escherichia coli* strain BL21 (DE3) cells were transformed with positive recombinant plasmid and cultured in LB broth with 50 mg l^{-1} kanamycin. Cells were grown at

Table 1
Summary of data-collection statistics.

Values in parentheses are for the last resolution shell.

Space group	$P2_12_12_1$
Unit-cell parameters (Å)	
a	38.98
b	43.94
c	78.17
Molecules in ASU	1
Resolution range	39.09–2.60 (2.69–2.60)
Total No. of reflections	38626
No. of unique reflections	4461
Completeness (%)	100.0 (100.0)
R_{merge}	0.125 (0.300)
Average $I/\sigma(I)$	11.2 (5.7)

310 K to an OD_{600} of 0.6 and were induced with 0.2 m*M* isopropyl *β*-D-1-thiogalactopyranoside (IPTG). The culture was further incubated for another 4 h for protein expression. The following procedure was performed at 277 K. The cells were harvested by centrifugation at 5000*g*, resuspended in affinity buffer *A* (50 m*M* sodium phosphate buffer pH 8.0 containing 0.25 *M* NaCl and 10 m*M* imidazole) and lysed by sonication on ice. To collect the soluble supernatant, the sonicated cells were centrifuged at 18 000 rev min^{-1} for 30 min. Post-centrifugation, the clear supernatant was loaded onto a column containing Ni–NTA His-binding resin (Novagen) previously equilibrated with buffer *A* as used for resuspending the cells. To wash the nonspecifically bound protein from the column, an increased concentration of imidazole was used in buffer *B* (50 m*M* sodium phosphate buffer pH 8.0 containing 0.25 *M* NaCl and 20 m*M* imidazole). For the elution of target protein, buffer *C* (50 m*M* sodium phosphate buffer pH 8.0 containing 0.25 *M* NaCl and 250 m*M* imidazole) was used. To further purify the protein by FPLC, the eluted protein was first dialysed against buffer containing 20 m*M* Tris–HCl pH 8.0, 50 m*M* NaCl, 1 m*M* mercaptoethanol and 1 m*M* EDTA and concentrated to the desired concentration and volume using a 10 kDa molecular-weight cutoff Centricon (Amicon). The protein sample was loaded onto a Superdex 200 column with an Akta Purifier System (GE Healthcare). The purified protein was examined using SDS–PAGE (Laemmli, 1970) and was found to be nearly homogenous (Fig. 1).

2.2. Crystallization and X-ray diffraction analysis

Adopting the hanging-drop vapour-diffusion method and using Crystal Screen, Crystal Screen 2, PEG/Ion Screen, Index Screen and Salt Rx Screen (Hampton Research), initial crystallization screening was carried out at 291 K. By mixing 2 µl each of the crystallization solution and protein solution (at a concentration of 5, 10 or 15 mg ml^{-1}), drops were set up against 250 µl reservoir solution. Crystals were obtained in two conditions from Crystal Screen: (i) 0.1 *M* sodium HEPES pH 7.5, 0.8 *M* sodium phosphate monobasic monohydrate, 0.8 *M* potassium phosphate monobasic and (ii) 0.1 *M* Tris–HCl pH 8.5, 2.0 *M* ammonium phosphate monobasic. These conditions were further optimized by varying the concentrations of the contributing components, the pH and the temperature. After a period of one month, crystals of cubic shape were found in the crystallization drops. To differentiate between salt and protein crystals and to save resources in checking the crystals using the X-ray machine, a small amount of methylene green was added to each drop. The crystals acquired a green stain after a period of 3–4 h, confirming them to be protein crystals, and were used for X-ray diffraction analysis (Fig. 2*a*). Crystals obtained by optimization of the second condition were used for X-ray diffraction analysis owing to their better diffraction data.

Using the crystals from these drops, X-ray diffraction data were collected using an in-house X-ray source (Rigaku RA-Micro7 desktop rotating-anode X-ray generator with a Cu target operated at 40 kV and 20 mA) and an R-AXIS IV^{++} imaging-plate detector at a wavelength of 1.5418 Å. The crystals were first cryoprotected by soaking them for a short period of time in a solution consisting of 50% each of the crystal-growth reservoir solution and glycerol. The crystal was flash-cooled in a cold nitrogen-gas stream at 100 K and data were collected. The data were analysed using *DENZO* and *SCALEPACK* from the *HKL*-2000 suite (Otwinowski & Minor,

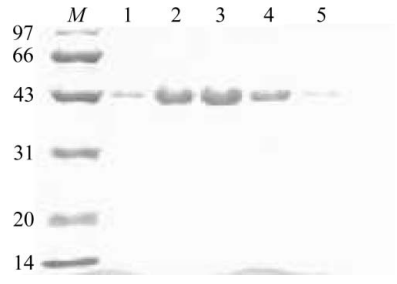
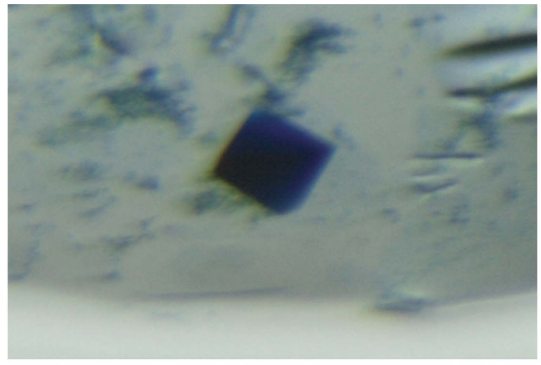
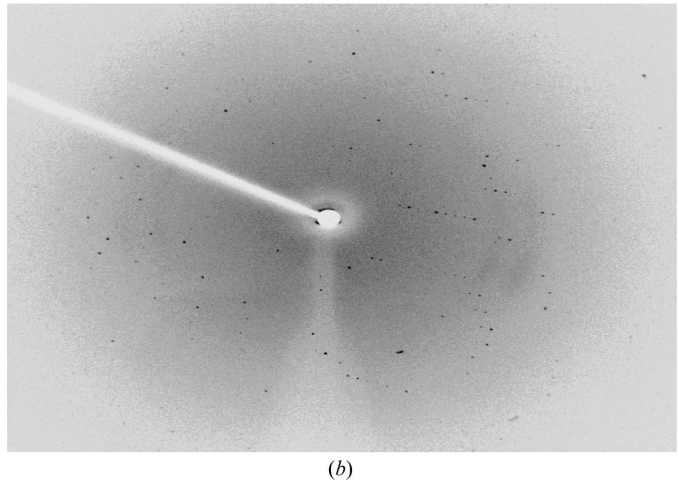


Figure 1
SDS–PAGE of IgGBP. The protein was analyzed on 12% SDS–PAGE and stained with Coomassie blue. Lanes 1, 2, 3, 4 and 5 contain purified His-tagged IgGBP fractions after size-exclusion chromatography; lane *M* contains molecular-weight markers labelled in kDa.



(*a*)



(*b*)

Figure 2
(*a*) Single crystal of IgGBP grown by the hanging-drop method. (*b*) Diffraction pattern obtained from an IgGBP crystal.

1997) and had a completeness of 100% to 2.6 Å resolution. Data-collection statistics are given in Table 1.

Molecular-replacement methods of phase determination were attempted, but the identity and similarity of the searched model to known structures was very low; therefore, selenomethionine-labelled protein has been expressed and crystallization is in progress. Unpublished functional data regarding the binding activity of this protein is in agreement with previous work.

This work was completed in the laboratory of Professor George F. Gao at the Institute of Microbiology, Chinese Academy of Sciences (IMCAS) and was supported by the Higher Education Commission of Pakistan and the Chinese Academy of Sciences.

References

Balber, A. & Sturtevant, J. (1986). *Infect. Immun.* **53**, 420–426.
Bessen, D. & Fischetti, V. (1990). *J. Infect. Dis.* **161**, 747–754.
Blumenfeld, W., Mandrell, R., Jarvis, G. & Griffis, J. M. (1990). *Infect. Immun.* **58**, 456–463.
Boyle, M. D. P. & Borsos, T. (1983). *The Reticuloendothelial System: A Comprehensive Treatise*, Vol. 4, edited by N. R. Rose & B. V. Siegel, pp. 43–76. New York: Plenum Press.
Bricker, B., Tabatabai, L. & Mayfield, J. (1991). *Mol. Immunol.* **28**, 35–39.
Ferher, S., Boyle, M. D. P. & Halliwell, R. (1988). *J. Vet. Res.* **49**, 697–701.
Laemmli, U. K. (1970). *Nature (London)*, **227**, 680–684.
Miller, J., Mekalanos, J. & Falkow, S. (1989). *Science*, **243**, 916–922.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Stannard, L. & Hardie, D. (1991). *J. Virol.* **65**, 3411–3415.
Underdown, B. J. & Schiff, J. M. (1986). *Annu. Rev. Immunol.* **4**, 389–417.
Widders, P. R., Dorrance, L. A., Yarnall, M. & Corbeil, L. B. (1989). *Infect. Immun.* **57**, 639–642.