Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary crystallographic analysis of BbCRASP-1, a complement regulator-acquiring surface protein of *Borrelia burgdorferi*

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Received 5 February 2004
Accepted 2 March 2004

Borrelia burgdorferi is the causative agent of Lyme disease. Serum-resistant strains of the pathogen are able to reduce the host's immune response to infection by recruiting fluid-phase complement regulators from the serum. *B. burgdorferi* complement regulator-acquiring surface protein-1 (BbCRASP-1) binds factor H and factor-H-like protein-1 to the bacterial surface, where they actively down-regulate complement response. Crystals of native and selenomethionine-substituted BbCRASP-1 have been obtained and a native data set to 2.7 Å as well as selenomethionine MAD data to 3.2 Å resolution have been collected. The selenium substructure has been solved and initial phases have been refined to 3.0 Å by density-modification methods. Model building and refinement are under way.

1. Introduction

Lyme borreliosis is the most frequent vector-borne disease in Eurasia and North America (Steere, 1989; Wang *et al.*, 1999), with approximately 75 000 new cases per year (O'Connell *et al.*, 1998; CDC, 2002). The classic manifestation of the disease is a temporary skin rash following initial infection which resolves spontaneously, although a wide range of other symptoms can occur (Stanek & Strle, 2003). Sporadically, the disease develops into a chronic multisystemic disorder that involves the skin, the nervous system and the joints (Lyme arthritis).

Lyme borreliosis develops in the human host as a consequence of bacterial infection with *Borrelia* (Burgdorfer *et al.*, 1982; Johnson *et al.*, 1984; Baranton *et al.*, 1992). The thin and elongated motile spirochaetes (Barbour & Hayes, 1986) are typically transmitted to the human host during a blood meal of infected *Ixodes* ticks, when the microbes gain access to the host dermis (Steere, 1989).

In the course of evolution *Borrelia* has acquired many sophisticated means of evading the host's immune defences, in particular attacks from the complement system that responds directly and immediately to the presence of the bacteria (Beutler, 2004). Along with other pathogens [*e.g.* *Streptococcus pyogenes* (Johnsson *et al.*, 1998; Kotarsky *et al.*, 1998; Perez-Caballero *et al.*, 2000), *S. pneumoniae* (Neeleman *et al.*, 1999), *Neisseria meningitidis* (Ram *et al.*, 1999), *N. gonorrhoeae* (Ram, McQuillen *et al.*, 1998; Ram, Sharma *et al.*, 1998), *Echinococcus granulosus* (Diaz *et al.*, 1997), *Yersinia enterocolitica* (China *et al.*, 1993) and the human immunodeficiency virus (Stoiber *et al.*, 1995)], serum-resistant strains of *Borrelia* are able to recruit the fluid-phase regulatory proteins factor H and/or factor-H-like protein-1 (FHL-1) from the serum (Kraiczy *et al.*, 2002). When bound to the outer membrane of the pathogen, these complement-control proteins down-regulate the host's complement response by rapidly inactivating newly formed C3b directly on the bacterial surface (Zipfel *et al.*, 1999). Previous studies provide direct evidence that a pathogen's ability to recruit host regulators depends on the expression and display of specialized surface proteins [*e.g.* porin for *N. gonorrheoeae* (Ram, McQuillen *et al.*, 1998; Ram, Sharma *et al.*, 1998), M protein for *S. pyogenes* (Ram *et al.*, 1999), Hic protein for *S. pneumoniae* (Janulczyk *et al.*, 2000; Jarva *et al.*, 2002) and gp120/gp41 of human immunodeficiency virus (Stoiber *et al.*, 1995)]. In the case of *Borrelia*, the presence of complement regulator-acquiring surface proteins (CRASPs) has been shown to be a requirement for complement evasion and serum-resistance (Kraiczy *et al.*, 2001).

Here, we report the expression, purification and preliminary crystallographic analysis of a member of the CRASP protein family, BbCRASP-1 from *B. burgdorferi*. BbCRASP-1 is a 28 kDa surface lipoprotein encoded on the linear plasmid lp54 together with ospA/B and dbpA/B. The predicted signal peptide and lipidation sequence (corresponding to amino acids 1–25 of the full-length sequence) was omitted from the recombinant construct in order to generate a soluble form of the protein. The primary sequence of BbCRASP-1 bears no significant sequence identity to any protein of known structure (sequence-similarity search

with BLAST; http://www.ebi.ac.uk/blastall/; Altschul *et al.*, 1997).

Recently, BbCRASP-1 has been shown to be the dominant factor H and FHL-1 binding protein of *B. burgdorferi*. Factor H and FHL-1 complement-regulating activities are retained upon binding to *Borrelia* and complement resistance of the bacteria directly correlates with expression of BbCRASP-1 (Kraiczy *et al.*, 2004).

As CRASPs are among the important determinants of the pathogenicity of *Borrelia*, obtaining structural data for a member of this protein family is an important first step towards a detailed understanding of the molecular basis for binding of host complement regulators by pathogens, with potential implications for the development of new drugs and treatment strategies.

2. Expression and purification of native and selenomethionine-labelled BbCRASP-1

A recombinant form of BbCRASP-1 in which the N-terminal region (amino acids 1–25) has been removed was produced and purified. Briefly, BbCRASP-1 was expressed as a glutathione *S*-transferase (GST) fusion protein (GST-BbCRASP-1) from the pGEX-2T (Amersham Biosciences) expression system in *Escherichia coli* JM109 (Promega) and purified as described previously (Kraiczy *et al.*, 2004). The fusion protein (GST-BbCRASP-1) was purified from bacterial lysates by affinity chromatography using glutathione Sepharose 4B; thrombin was used to separate BbCRASP-1 from the GST tag. Yields of ∼3 mg of 99% pure BbCRASP-1 per litre of bacterial culture could be obtained. Recombinant BbCRASP-1 lacks the hydrophobic leader sequence and signal peptidase II site of the native protein precursor (Kraiczy *et al.*, 2004). Gly-Ser replaces the mature native lipidated N-terminal Cys25; the remainder of the recombinant polypeptide follows the BbCRASP-1 sequence. The predicted weight of the expressed fragment is 26 381 Da. The purified protein was buffer-exchanged into 10 m*M* Tris–HCl pH 7.4 over three 1/20 dilution/concentration cycles and concentrated to a final concentration of 38 mg ml^{-1} (as judged by OD_{280nm} readings using the predicted extinction coefficient of 12 800 M^{-1} cm^{-1}; http://us.expasy.org/tools/protparam.html; Gill & von Hippel, 1989) in Centricon concentrators (Millipore UK Ltd, Watford, UK) with a molecular-weight cutoff of 10 kDa. Aliquots of the final sample were stored at 277 K for direct use in crystallization or frozen at 193 K for later use.

Selenomethionylated (SeMet) GST-BbCRASP-1 was expressed in *E. coli* B834 (DE3) by standard methods (Davies *et al.*, 2000) and purified using the same procedure as described for the native protein. Approximately 2.2 mg of SeMet-labelled BbCRASP-1 was purified from 1 l of culture. An SDS–PAGE analysis of 1.5 µl protein is shown in Fig. 1. As for the native protein, SeMet BbCRASP-1 was concentrated to ∼38 mg ml^{-1} in 10 m*M* Tris–HCl pH 7.4 and stored in small aliquots at 193 K.

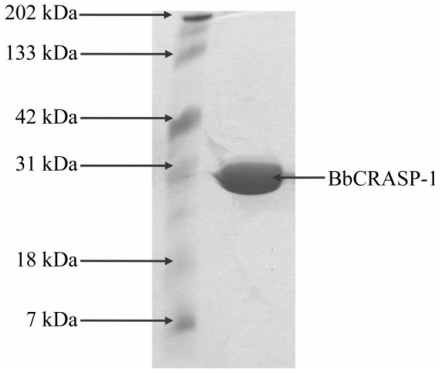

Figure 1
SDS–PAGE analysis of purified BbCRASP: 1.5 µl of protein sample prior to the concentration step analysed on 15% SDS–PAGE gel.

3. Crystallization of native and selenomethionine-labelled BbCRASP-1

Crystallization conditions were searched by sparse-matrix screening (Jancarik & Kim, 1991) using the vapour-diffusion technique at 293 K in sitting drops with a volume of 1 µl protein plus 1 µl mother liquor. Initially, a total of 120 conditions were screened using Molecular Dimensions Screens 1 and 2 (Molecular Dimensions Ltd, Soham, UK) and a PEG Screen as described by Stura *et al.* (1994), followed by a series of customized fine grid screens at 285 and 293 K around favourable conditions from the initial screens using a Tecan crystallization robot (Tecan UK, Theale, UK). Rod-shaped crystals of native BbCRASP-1 with approximate dimensions 100 × 100 × 600 µm (Fig. 2) were obtained from the 38 mg ml^{-1} frozen protein stock using 25% PEG 600, 200 m*M* imidazole at pH 6 as the precipitant at 293 K. These crystals proved to be suitable for X-ray diffraction analysis. SeMet BbCRASP-1 crystals were grown using a protein solution of identical composition and concentration to that for the native protein and crystallized using the same precipitant solution as used for the native with the addition of 5% glycerol.

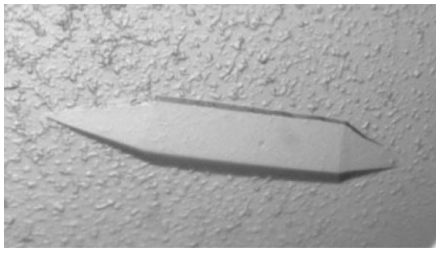

Figure 2
Crystal of BbCRASP-1 obtained by sitting-drop vapour diffusion. The crystal measures approximately 600 µm in length.

4. Data collection

Crystals were briefly washed in 35% PEG 600, 200 m*M* imidazole pH 6.0 for cryoprotection and were then cryocooled in liquid nitrogen. Initial tests were performed using a rotating-anode generator and yielded diffraction to 4 Å.

The diffraction pattern revealed that the unit-cell parameters are $a = b = 90$, $c = 146$ Å, with systematic absences characteristic of space group $P4_32_12$ or $P4_12_12$. Consideration of the Matthews coefficient (Matthews, 1968) and the protein size suggested that the asymmetric unit was likely to contain two copies of BbCRASP-1 (Matthews coefficient of 3.0 Å^3 Da^{-1}), although three copies would also have been allowed (two copies give a solvent content of 59% and three copies 38%; Collaborative Computational Project, Number 4, 1994). Higher resolution data sets were collected under cryoconditions at 100 K at the European Synchrotron Radiation Facility (ESRF, Grenoble, France). A 2.7 Å native data set was recorded at beamline ID 14.2. Crystals of SeMet-labelled BbCRASP-1 showed a well defined Se *K* absorption edge by fluorescence scanning (Fig. 3) and data sets were collected from these crystals at beamline ID 29 at a single wavelength (B12-17) and at two wavelengths [peak (C10-pk) and low-energy remote (C10-remo)] for MAD phasing. Both native and SeMet crystals showed anisotropic diffraction, with the resolution generally limited to ∼3 Å in the most favourable portions of reciprocal space. All samples suffered significantly from radiation damage upon repeated exposure, which proved to be a particular limitation in collection of data from SeMet-labelled crystals.

All data were indexed and integrated using *MOSFLM* (Leslie, 1992) and scaled with anisotropic scaling corrections using *SCALA* (Evans, 1993) within the *CCP*4 suite (Collaborative Computational Project,

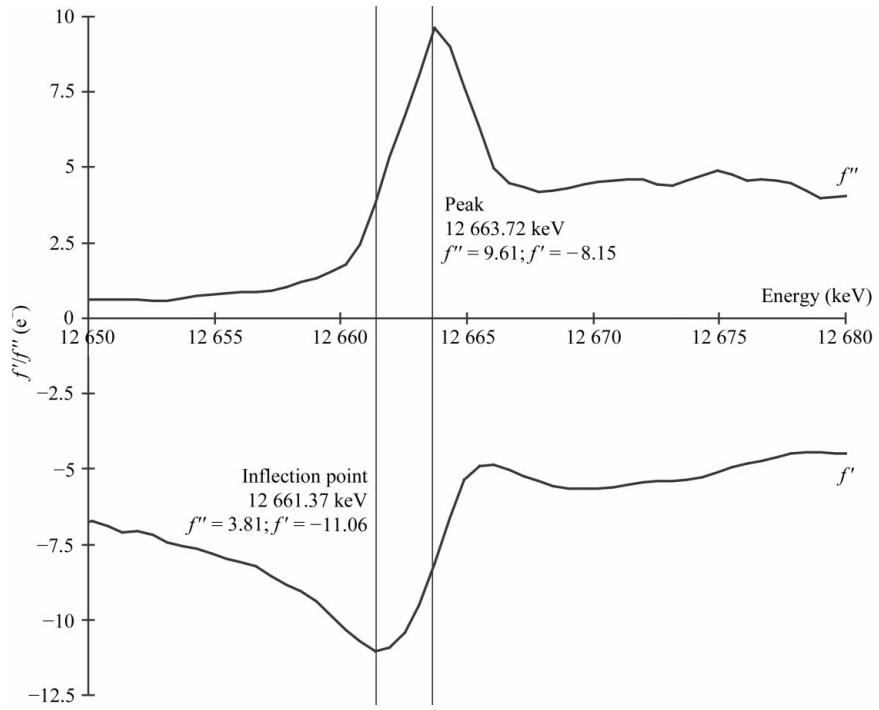

Figure 3
Anomalous and dispersive Se scattering factors across the *K* edge derived from a fluorescence scan.

Table 1
Data-collection statistics for BbCRASP-1.

Values in parentheses correspond to the highest resolution shell.

Data set	Native (Nat3)	SeMet (B12-17)	SeMet (C10-remo)	SeMet (C10-pk)
Wavelength (Å)/(keV)	0.934/13274	0.979/12664	0.984/12600	0.979/12664
Unit-cell parameters (Å)				
$a = b$	90	92	92	92
c	146	146	147	147
Resolution (Å)	56.5–2.7 (2.85–2.7)	72–3.2 (3.37–3.2)	73–3.2 (3.37–3.2)	73.3–3.2 (3.37–3.2)
Unique reflections	13090	10858	10844	10849
Multiplicity	2.4 (2.4)	7.4 (7.8)	7.5 (7.8)	7.6 (7.9)
Completeness (%)	95.0 (97.6)	99.7 (100)	100 (100)	100 (100)
$R_{\rm merge}$† (%)	4.8 (25.7)	10.1 (44.1)	9.9 (47.1)	8.8 (42.0)
$I/\sigma(I)$	9.1 (3.0)	4.0 (1.7)	4.8 (1.6)	4.0 (1.8)
$R_{\rm anom}$‡ (%)	n.a.	5.2 (20.8)	n.a.	5.5 (19.6)

† $R_{\rm merge} = 100 \times [\sum_h \sum_i |\langle I(h)\rangle - I(h)_i| / \sum_i I(h)_i]$, where $I(h)_i$ is the ith observation of reflection h and $\langle I(h)\rangle$ is the mean intensity of all observations of h. ‡ $R_{\rm anom} = 100 \times \sum_h |\langle I^+\rangle - \langle I^-\rangle| / \sum_h (\langle I^+\rangle + \langle I^-\rangle)$, where $\langle I^+\rangle$ and $\langle I^-\rangle$ are the mean intensities of the Bijvoet pairs for observation h.

Number 4, 1994). Processing statistics for the best native and SeMet data sets are shown in Table 1. The unit-cell parameters vary marginally between crystals of the same sample and are slightly smaller for the unlabelled protein.

5. Selenium-substructure solution and 3.2 Å phasing

The selenium substructure was solved with the program *HySS* (Adams *et al.*, 2002; Grosse-Kunstleve & Adams, 2003) using the SeMet data (C10-peak) described in Table 1 and limiting the resolution to 3.5 Å. Assuming two copies of BbCRASP-1 within the asymmetric unit, there are six potential methionines (plus two N-terminal methionines). *HySS* produced a clear solution locating four Se atoms within the asymmetric unit, suggesting that it contains two BbCRASP-1 molecules but that at least two methionine residues do not contribute to the anomalous signal. Initial phases were generated in *SHARP* (de la Fortelle & Bricogne, 1997), refined at a resolution of 3.2 Å, giving a figure of merit of 0.31 for centrics and 0.20 for acentrics. These phases were then solvent-flattened to 3.0 Å using *SOLOMON* (Abrahams & Leslie, 1996) in both hands. This demonstrated that the correct space group was $P4_32_12$ [correlation coefficient on $|E|^2$ after solvent flattening is 58% in $P4_32_12$, 49% in $P4_12_12$; figures of merit in the low-resolution bins (data to 10 Å) at the same stage are 0.84 for $P4_32_12$ and 0.66 for $P4_12_12$]. The maps produced provide clear evidence of two copies of a mainly α-helical molecule, but are not amenable to automatic tracing and model building. Phase extension to the full resolution of the native data set was not successful, presumably owing to the lack of isomorphism between the native and SeMet crystals.

6. Discussion

The current solvent-flattened phases should allow construction of a model for BbCRASP-1 and the 2.7 Å data should enable a careful refinement of the model constructed (Table 1). Analytical ultracentrifugation will be used to determine whether BbCRASP-1 is a dimer in solution and mutagenesis will be used to test functional hypotheses concerning a physiological role for the dimer.

We would like to thank R. Ravelli for his assistance at the ESRF beamline ID 29. M. E. M. Noble and J. Welburn are thanked for collecting the native data set described in Table 1. FSC is funded by a Wellcome Trust studentship (No. 059380 to SML) and PR by the BBSRC (grant No. 43/B16601 to SML).

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