Complement Resistance of *Borrelia burgdorferi* Correlates with the Expression of BbCRASP-1, a Novel Linear Plasmid-encoded Surface Protein That Interacts with Human Factor H and FHL-1 and Is Unrelated to Erp Proteins*

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The etiologic agent of Lyme disease, *Borrelia burgdorferi*, is capable of circumventing the immune defense of a variety of potential vertebrate hosts. Previous work has shown that interaction of host-derived complement regulators, factor H and factor H-like protein 1 (FHL-1), with up to five complement regulator-acquiring surface proteins (CRASPs) expressed by resistant *B. burgdorferi* sensu lato isolates conferred complement resistance. In addition expression of CRASP-1 is directly correlated with complement resistance of *Borrelia* species. This work describes the functional characterization of BbCRASP-1 as the dominant factor H and FHL-1-binding protein of *B. burgdorferi*. The corresponding gene, zs7.a68, is located on the linear plasmid lp54 and is different from factor H-binding Erp proteins that are encoded by genes localized on circular plasmids (cp32). Deletion mutants of BbCRASP-1 were generated, and a high affinity binding site for factor H and FHL-1 was mapped to the C terminus of BbCRASP-1. Similarly, the predominant binding site of factor H and FHL-1 was localized to the short consensus repeat 7. Factor H and FHL-1 maintain their cofactor activity for factor I-mediated C3b inactivation when bound to BbCRASP-1, and factor H is up to 6-fold more efficient in mediating C3b conversion than FHL-1. In conclusion, BbCRASP-1 (i) binds the host complement regulators factor H and FHL-1 with high affinity, (ii) is the key molecule of the complement resistance of spirochetes, and (iii) is distinct from the Erp protein family. Thus, BbCRASP-1 most likely contributes to persistence of *B. burgdorferi* and to pathogenesis of Lyme disease.

Lyme borreliosis is a complex multisystemic disorder caused by pathogenic species of the *Borrelia burgdorferi* sensu lato complex and is regarded as the most frequent vector-borne infectious disease in North America and Europe (1). Because of the complex enzootic cycle of *B. burgdorferi* in nature including diverse environments such as arthropod vectors and a variety of vertebrate hosts, spirochetes have developed strategies to survive in both vector and reservoir hosts. These include their ability (i) to evade an immune defense by differential expression of polymorphic outer surface proteins (2), (ii) to sequester into immune privileged sites (3, 4), and (iii) to adapt to new environmental stimuli (5). One particular strategy of spirochetes involves their resistance to complement-mediated killing in the mammalian host (6–9).

The complement system forms an important part of the innate immunity and plays a crucial role in the elimination of invading microorganisms. Direct activation of complement via the alternative or the mannose-binding lectin pathway results in opsonization and formation of the lytic membrane attack complex leading to killing of the invading microorganisms (10). An increasing number of microorganisms pathogenic to humans, including *B. burgdorferi* (11–13), *Neisseria gonorrhoeae* (14), *Neisseria meningitidis* (15), *Streptococcus pyogenes* (16–19), and *Streptococcus pneumoniae* (20), resist complement-mediated killing by coating their surfaces with host-derived fluid phase negative complement regulators of the alternative pathway, factor H, and/or factor H-like protein 1 (FHL-1).1

Factor H and FHL-1 belong to a protein family that is structurally composed of individually folded protein domains, termed short consensus repeats (SCRs), or complement control protein modules (21, 22). Factor H consists of 20 SCR domains; and FHL-1, an alternatively spliced variant of the factor H gene, represents the first seven SCRs of factor H and includes an extension of four hydrophobic amino acids (SFTL) at its C terminus. Both plasma proteins control the alternative pathway of complement activation at the level of C3b by competing with factor B for binding to C3b. These regulators accelerate the decay of the C3 convertase, C3bBb (decay-accelerating activity), and act as cofactors for factor I-mediated degradation of C3b (23–26).

1 The abbreviations used are: FHL-1, factor H-like protein 1; CRASPs, complement regulator-acquiring surface proteins; SCRs, short consensus repeats; GST, glutathione S-transferase; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; NHS, Non-im- mune human serum; mAb, monoclonal antibody; BbCRASPs, complement-resistant *B. burgdorferi*; BaCRASPs, complement-resistant *B. afzelii*; aa, amino acids.


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Several pathogenic microorganisms express surface proteins capable of interacting with factor H and FHL-1, such as the M- and the Fba protein of S. pyogenes (16, 27, 28). PspC, Hic proteins of pneumococci (20, 29–31), PorA protein of nonsialylated N. gonorrhoeae (32), and envelope proteins gp41 and gp120 of the human immunodeficiency virus (33). Our previous studies indicated that moderate or fully complement-resistant B. burgdorferi ZS7 genomic DNA expression library was prepared and screened using recombinantly expressed FHL-1 and factor H deletion (12, 37 polymorphic Erp (OspE/F-related proteins) family (35). A molecular level and identified as a novel member of the BbCRASPs) and FHL-1-binding proteins (BaCRASP-3), and factor H-binding (BaCRASPs) strains are divided into three groups: factor H- and FHL-1-binding proteins (BbCRASP-1, BaCRASP-1, BbCRASP-2, and BaCRASP-2), FHL-1-binding proteins (BaCRASP-3), and factor H-binding proteins (BbCRASP-3–5, BaCRASP-4, and BaCRASP-5) (34, 36). More recently, BbCRASP-3 has been characterized on a molecular level and identified as a novel member of the polymorphic Erp (OspE/F-related proteins) family (35). BbCRASP-3 and multiple homologous Erp proteins expressed by virulent B. burgdorferi strains were shown to bind factor H (12, 37–40). In addition, we found that CRASPs comprise at least two different groups of outer surface proteins, one of which is represented by the Erp protein family (35).2

The purpose of the present study was to identify and characterize BbCRASP-1, a novel outer surface protein of B. burgdorferi that is different from the Erp protein family and represents the predominant factor H- and FHL-1-binding protein.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains, Culture, and Materials—**Intermediate complement-resistant B. burgdorferi strain ZS7 (tick isolate, Germany), infectious and pathogenic in mice, was grown at 33 °C for 5–6 days up to a cell density of $1 \times 10^7$/ml in modified Barbour-Stoenner-Kelly (BSK) medium as described previously (35). Cells were harvested by centrifugation at 5000 rpm for 30 min and resuspended in sterile phosphate-buffered saline (PBS) (140 mM NaCl, 2.7 mM KCl, 10 mM Na$_2$HPO$_4$, 1.8 mM KH$_2$PO$_4$, pH 7.3) containing 5 mM MgCl$_2$ (PBS-Mg). The density of spirochetes was determined using dark-field microscopy and a Kova counting chamber (Hycor Biomedical, Garden Grove, CA). Escherichia coli MC1061, JM109, and DH5α were grown at 37 °C in LB or 2× YT medium.

**Preparation and Screening of a B. burgdorferi Expression Library—**A B. burgdorferi ZS7 genomic DNA expression library was prepared and screened using recombinantly expressed FHL-1 and factor H deletion constructs as described previously (35, 41). Briefly, bacterial colonies were plated onto LB agar plates and transferred to nitrocellulose filters. Membranes were incubated with supernatant of S9 cells infected with FHL-1 or various recombinant deletion constructs of factor H (FH1–12, FH1–20, FH1–20, FH19–20) for 12 h at 4 °C. After three washings with TBS containing 0.2% Tween 20, filters were incubated with antisera to SCR1–4 (24) and to SCR20 (VIGS) (42), specific for factor H/FHL-1 and factor H, respectively, in the presence of 1% MC1061 cell lysate, followed by incubation with the appropriate peroxidase-conjugated secondary antibody.

**Construction of Expression Plasmids and Purification of Recombinant Borrelial Proteins—**The BbCRASP-1 gene was subcloned by PCR using plasmid pUEX15, and the amplified DNA fragment, previously named z7a68, was ligated in-frame into vector pGEX-2T which includes the glutathione S-transferase gene at the N terminus of the expressed recombinant fusion protein (41). Expression of the GST-BbCRASP-1 fusion protein in E. coli JM109, affinity purification on glutathione-Sepharose columns, and endoprotease thrombin cleavage of the glutathione S-transferase (GST) fusion protein was performed as recommended by the manufacturer (Amersham Biosciences). C-terminal deletion mutants of BbCRASP-1 were constructed by PCR using the pGEX sequencing primer in combination with oligonucleotides BbCRASP-1/313–5, BbCRASP-1/490–5, BbCRASP-1/587–5, BbCRASP-1/709–7, or BbCRASP-1/1708–7, respectively. Oligonucleotides used for PCR as listed in Table I were purchased from Sigma or Roth (Mannheim, Germany). The amplified DNA fragments were digested with BamHI and ligated in-frame with the glutathione S-transferase gene into the pGEX-2T vector (Amersham Biosciences) resulting in plasmids pGEX ZSA68/13, pGEX ZSA68/490, pGEX ZSA68/837, pGEX ZSA68/709, and pGEX ZSA68/730. Expression of the GST fusion proteins in E. coli DH5α and affinity purification were performed according to the instructions of the manufacturer (Amersham Biosciences). Expression and purity of all GST fusion proteins were confirmed by employing Tris/Tricine-SDS-PAGE (34, 35), and protein concentrations were determined by a Bradford assay (Bio-Rad).

**Expression of Recombinant Proteins of Factor H and FHL-1—**FHL-1 and deletions constructs of factor H (FH1–2, FH1–3, FH1–4, FH1–5, FH1–6, FH1–20, FH15–20, and FH19–20) were expressed in S9 insect cells infected with recombinant baculovirus. The cloning of various deletion constructs, expression, and purification have been described previously (24, 43, 44).

**DNA Sequence Analysis—**B. burgdorferi genomic DNA fragments cloned in pUBE1 or pGEX-2T plasmid derivatives were sequenced by using the BigDye Terminator Cycle sequencing kit (PE Applied Biosystems, Foster City, CA) in accordance with the manufacturers’ recommendations.

** SDS-PAGE, Western Ligand Affinity Blots, and Western Blot—**Cell lysates or purified recombinant proteins were subjected to Tris/Tricine-SDS-PAGE (reducing conditions), transferred to nitrocellulose, and probed with the corresponding antisera as described previously (34, 35).Surface Plasmon Resonance Assays—Protein-protein interactions were analyzed by surface plasmon resonance technique using a Biacore 3000 instrument (Biacore AB, Uppsala, Sweden) as described earlier (35, 44). Briefly, borrelial recombinant proteins BbCRASP-1 or C-ter-
nal deletion mutants (BbCRASP-1-[26–244], BbCRASP-1-[26–240], BbCRASP-1-[26–166], and BbCRASP-1-[26–108]) (20 μg/ml, dialyzed against 10 mM acetate buffer, pH 5.5) were coupled via a standard amine-coupling procedure to the flow cells of a sensor chip (CM5, Biacore AB) until a level of >4,000 resonance units was reached. A control flow cell was prepared in the same way but without injecting a protein. Factor H, FHL-1, and deletion construct FH1–6 were dialyzed against running buffer (75 mM phosphate-buffered saline, pH 7.4). Each ligand (factor H, 335 μg; FHL-1 and FH1–6, 1 μg) was injected separately into the flow cell coupled with BbCRASP-1 or the deletion mutants and into a control flow cell using a flow rate of 5 μl/min at 25 °C. Each interaction was analyzed at least three times.

The binding kinetics were determined using a lower density of the immobilized ligand (<1,000 resonance units) at 22 °C in 75 mM phosphate-buffered saline, pH 7.4, and employing a natural logarithmic Langmuir 1:1 binding model and the simultaneous Kₐ/Kᵦ fitting routine of the BIAevaluation 3.1 software (Biacore, AB). The equilibrium constants were calculated from the rate constants.

PepSpot Analysis—A library of 72 peptides, representing the entire BbCRASP-1 protein, SCR7 of factor H/FHL-1, and SCR19–20 of factor H was synthesized and spotted on a cellulose membrane (Jerini Peptide Technologies, Berlin, Germany). Each peptide was 13 amino acids in length and differed from the next peptide in 3 amino acid residues. Therefore, each peptide overlapped with the next by 10 amino acids. Membranes were incubated with recombinant proteins, and binding was detected with specific antibodies directed against the N-terminal 15 amino acids of factor H. Sera that proved negative for anti-BbCRASP-1 and mAb RH1 directed against BbCRASP-1 and mAb N38 1.1 directed against BbCRASP-3 protein, respectively. Three putative factor H-binding sites were localized specifically reactive with both complement regulators, factor H and FHL-1. Two regions of BbCRASP-1, aa 202–215 and aa 154–174, and the terminal 11 amino acid residues of BbCRASP-1 are relevant for binding of both factor H and FHL-1.

These results were further confirmed by surface plasmon resonance analysis (Fig. 3). Consistent with the ligand affinity blot analyses, binding of the full-length form of BbCRASP-1 to both factor H and FHL-1 was readily observed and was dramatically reduced when the various deletion mutants were employed. BbCRASP-1-[26–244] and BbCRASP-1-[26–240] showed reduced binding of factor H, whereas BbCRASP-1-[26–166] and BbCRASP-1-[26–108] completely abolished binding of factor H (Fig. 3A). Similarly, FHL-1 showed reduced binding to BbCRASP-1-[26–244], whereas no binding to any of the other deletion mutants of BbCRASP-1. As positive control for factor H binding, recombinant BbCRASP-3 was included (Fig. 2D, lane 7). These data indicate that the C-terminal 11 amino acid residues of BbCRASP-1 are relevant for binding of both factor H and FHL-1.

RESULTS

Identification and Cloning of the Gene Encoding BbCRASP-1 of B. burgdorferi Strain ZS7—In order to identify B. burgdorferi CRASP-1, a genomic DNA expression library derived from B. burgdorferi strain ZS7 was screened for factor H and FHL-1-binding clones. From 15 clones initially identified, one was particularly reactive with both complement regulators, factor H and FHL-1. Sequence analysis of this clone revealed that the open reading frame was identical to the zs7.a68 gene described previously (41). Comparative sequence analysis showed that the zs7.a68 gene was homologous to bbo68 of strain B31 (47). Pulsed field gel electrophoresis demonstrated that zs7.a68 is located together with ospA/B and dbpA/B on the 54-kb linear plasmid of B. burgdorferi ZS7 (41). zs7.a68 encodes a unique protein with a calculated molecular mass of 28 kDa, and is hereafter termed BbCRASP-1. The predicted N terminus of BbCRASP-1 shows significant homology to the signal peptides of other bacterial lipoproteins. This motif includes three lysine residues near the N terminus, a hydrophobic region, and a sequence similar to the consensus signal peptide II cleavage sequence LX₁₋₄XC. Subsequent lipidation at the cysteine residue 25 predicts BbCRASP-1 as an outer surface lipoprotein of B. burgdorferi (Fig. 1).

Localization of the Factor H and FHL-1-Binding Site within BbCRASP-1—In order to localize the factor H and FHL-1-binding site within BbCRASP-1, we initially searched the sequence of BbCRASP-1 for factor H-binding motifs similar to that previously identified in BbCRASP-3 (AJ508772) (35), LEVLKKNLK, by using the DNAstar Lasergene 99 software package. Three putative factor H-binding sites were localized within the middle and the C-terminal region of BbCRASP-1, i.e. regions 1–3 encompassing an 145–154, 204–213, and 233–242, respectively (Fig. 1). Next, we generated various C-terminal truncations of BbCRASP-1 (Fig. 2A). Screening for factor H and FHL-1 binding, employing several recombinant and purified proteins as depicted in Fig. 2B, revealed that only the full-length form of BbCRASP-1 and deletion mutant BbCRASP-1-[26–244] bound to FHL-1 (Fig. 2C). Deletion mutant BbCRASP-1-[26–244] exhibited reduced signal intensity in this assay compared with recombinant BbCRASP-1. In addition, binding of factor H was only observed with full-length BbCRASP-1 and BbCRASP-3 (Fig. 2D). No binding to factor H was detected with deletion mutant BbCRASP-1-[26–244] nor with any other deletion mutant of BbCRASP-1. As positive control for factor H binding, recombinant BbCRASP-3 was included (Fig. 2D, lane 7). These data indicate that the C-terminal 11 amino acid residues of BbCRASP-1 are relevant for binding of both factor H and FHL-1.

by having demonstrated that the amino acid residues located at the C terminus of BbCRASP-1 are necessary for factor H and FHL-1 binding, we assessed by pepspot analysis whether additional regions of linear epitopes do interact with factor H and FHL-1. Two regions of BbCRASP-1, aa 202–218
and 236–248, were observed that showed positive signals in this assay (Fig. 4). The region harboring amino acid FNKYYKDFDTLKP (aa 236–248) overlapped with the C-terminal region 3 (aa 233–242) (Fig. 1), and a second region encompassing aa 208–218 (SNLEIQRLKKTLNETL) is identical to the proposed factor H-binding region 2 (aa 204–213) as depicted in Fig. 1. Our analysis thus identifies in addition to the C-terminal region a further linear domain in BbCRASP-1 that is involved in binding of factor H and/or FHL-1.

Protease Accessibility and Surface Localization of BbCRASP-1—in order to test protease accessibility of BbCRASP-1 as a measure of surface localization, Western blot analyses were performed with in situ digested viable B. burgdorferi using protease K or trypsin. As shown in Fig. 5A, a significant reduction could be observed for BbCRASP-1 and BbCRASP-3 after 2 h of incubation with protease K at concentrations ≥12.5 μg/ml. In contrast, incomplete degradation was detected for OspA and OspB. The limited accessibility of OspA and OspB of B. burgdorferi strain ZS7 to protease K is reminiscent of previous reports (48) using different B. burgdorferi strains. No change in band intensity was observed for flagellin and Hsp70, which are localized to the periplasmic space and inside the protoplasmic cylinder of spirochetes. Upon treatment of trypsin, a more site-specific protease, BbCRASP-1 was degraded at concentrations ≥12.5 μg/ml, whereas higher amounts (>50 μg/ml) of the same protease were required for almost complete degradation of BbCRASP-3 and OspB. In contrast, OspA, flagellin, and Hsp70 were resistant to trypsin even at the highest concentration applied. Thus, protease susceptibility of BbCRASP-1 indicates that this protein is exposed at the outer surface of B. burgdorferi strain ZS7.

In a further approach the influence of protease treatment on the binding capacity of factor H to BbCRASP-1 and BbCRASP-3 was analyzed by ligand affinity blotting. As shown in Fig. 5B, binding of factor H to BbCRASP-1 and to BbCRASP-3 could be detected when untreated protein prepa-
**FIG. 2. Localization of the factor H- and FHL-1-binding region in the BbCRASP-1 protein.** A, diagrammatic representation of native and expressed recombinant BbCRASP-1 proteins. The numbers refer to amino acid residues, and the leader sequence is indicated. B, Coomassie stain of purified recombinant proteins separated by 10% Tris/Tricine gels. Lane 1, GST-BbCRASP-1. Lane 2, deletion mutant GST-BbCRASP-1-(26–108). Lane 3, deletion mutant GST-BbCRASP-1-(26–166). Lane 4, deletion mutant GST-BbCRASP-1-(26–215). Lane 5, deletion mutant GST-BbCRASP-1-(26–240). Lane 6, deletion mutant GST-BbCRASP-1-(26–244). Lane 7, GST-BbCRASP-3. Lane 8, purified GST. C, ligand affinity blot analysis to detect FHL-1 binding. The membrane was incubated with the cell supernatant of FHL-1, and binding was detected using oSCR1–4 as detecting antibody. D, ligand affinity blot analysis to detect factor H binding. The membrane was incubated with NHS, and binding of factor H to recombinant BbCRASP-1 and the various deletion mutants was detected by using monoclonal antibody VIG8 specific for SCR20 of factor H. The mobility of marker proteins is indicated on the left.
visions were used but was completely abrogated upon treat-
ment with proteinase K (12.5 μg/ml) or trypsin (25 μg/ml).

Taken together, susceptibility of BbCRASP-1 to protease cleav-
age with subsequent loss of factor H binding suggested that
this protein is localized to the outer cell compartment of
B. burgdorferi.

Mapping of the BbCRASP-1-binding Site(s) of Factor H and
FHL-1—Previously, we have reported that binding of both fac-

**Fig. 3. Analysis of factor H and FHL-1 for binding to BbCRASP-1 deletion mutants by surface plasmon resonance.** Either factor H (333 nM) or FHL-1 (1 μM) in the fluid phase was injected into a flow cell precoupled with borrelial proteins and to a control flow cell without protein. The control was subtracted from the displayed binding curves. Binding of factor H to the complete BbCRASP-1 and to C-terminal deletion mutants BbCRASP-1-(26–244), BbCRASP-1-(26–240), BbCRASP-1-(26–215), BbCRASP-1-(26–166), and BbCRASP-1-(26–108) was measured. As compared with the native protein, binding to mutants BbCRASP-1-(26–244) and BbCRASP-1-(26–240) was dramatically reduced, and no binding was seen with mutants BbCRASP-1-(26–215), BbCRASP-1-(26–166), and BbCRASP-1-(26–108) (A). Binding of FHL-1 to the complete BbCRASP-1 and to the mutants of the protein was measured. Compared with the native protein, binding to mutant BbCRASP-1-(26–244) was reduced by more than 50%, whereas all the other mutants showed no binding in the assay (B).
factor H and FHL-1 to native BbCRASP-1 is predominantly mediated via SCR5–7, and in addition a weak binding of the C-terminal domains (SCR19–20) of factor H was observed (34). We now intended to map precisely the binding sites of factor H and FHL-1 to recombinant BbCRASP-1 of B. burgdorferi strain ZS7 by employing FHL-1 and various deletion constructs of factor H in combination with ligand affinity blotting techniques. As shown in Fig. 6, BbCRASP-1 strongly bound to FHL-1 (lane 7) and in addition deletion constructs FH1–6 (lane 6) and FH1–5 (lane 5) did bind but not to FH1–4 (lane 4), FH1–3 (lane 3), and FH1–2 (lane 2). Applying deletion constructs representing C-terminal SCRs of factor H, i.e. FH8–20, FH15–20, and FH19–20, show positive but weak binding to BbCRASP-1 (Fig. 6, lanes 8–10). These data indicate that B. burgdorferi BbCRASP-1 Interacts with Factor H and FHL-1
SCR5–7 of factor H and FHL-1 are critical for interaction with BbCRASP-1 and SCR19–20 contributes to this binding, however, with lower affinity. Furthermore, pepspot analysis indicated that a linear stretch of 16-aa residues of SCR7 (aa 398–413) and an 18-aa stretch of SCR20 (aa 1202–1217) participate in BbCRASP-1 binding (Fig. 6B). In contrast, assays using more physiological conditions, such as surface plasmon resonance and ELISA techniques, showed different results. Factor H and FHL-1 but none of the deletion constructs FH1–6, FH1–5, and FH19–20 bound to BbCRASP-1 (data not shown) suggesting that the physiologically important high affinity binding site is most likely localized to domain SCR7. By applying surface plasmon resonance analyses, both FHL-1 and factor H bound to immobilized BbCRASP-1 although with different affinities (Table II). Quantitative analysis showed that FHL-1 binds to BbCRASP-1 with a higher affinity as compared with factor H presumably due to the hydrophobic tail that is absent in factor H. The calculated $K_d$ values for factor H and FHL-1 are 28 and 12 nM, respectively.

Factor H and FHL-1 Retain Their Cofactor Activity When Bound to Recombinant BbCRASP-1—We hypothesized previously that coating of spirochetes with factor H and FHL-1 via BbCRASP-1 may play an important role for immune evasion of Borrelia. Therefore, functional activity of factor H and FHL-1 bound to recombinant BbCRASP-1 was tested for C3b inactivating capacity. When equinmolar amounts of factor H and FHL-1 were added to BbCRASP-1-coated microtiter plates about 10–20% more FHL-1 molecules were bound (data not shown). However, factor H bound to BbCRASP-1 was up to 6-fold more efficient in mediating C3b conversion than FHL-1 (Fig. 7). Addition of an unrelated recombinant protein (L1) to BbCRASP-1 had no effect on C3b conversion.

**DISCUSSION**

The present study describes the identification and functional characterization of a novel outer surface protein, termed BbCRASP-1, of *B. burgdorferi*, a key protein for complement resistance to spirochetes (13, 34). BbCRASP-1 is encoded by a linear plasmid lp54 localized gene and distinct from members of the factor H-binding Erp protein family. Functional analyses employing recombinant derivatives of BbCRASP-1 reveal that the major binding site for both factor H and FHL-1 is associated with an 11-amino acid domain at the C terminus of BbCRASP-1 and that the SCR7 domain of factor H and FHL-1 was essential for optimal binding. However, factor H showed a significant higher potential than FHL-1 to inactivate C3b.

BbCRASP-1 belongs to the paralogous family gbb54 of *B. burgdorferi* strain B31 with 14 members encoding yet hypothetical proteins with unknown function. In contrast to the *erp* genes that are localized to the cp32 family of plasmids and encode another family of factor H-binding proteins, *bbCRASP-1* (formerly named *zs7.a68*) is located together with the *ospA*, *ospB*, *dbpA*, *dbpB*, and *p35* on the linear plasmid lp54 of *B. burgdorferi* strain ZST (41). Protease susceptibility assays with intact, viable spirochetes identified BbCRASP-1 as a surface-exposed protein. The additional finding that BbCRASP-1 does not share any significant protein sequence homology with members of the Erp protein family indicates that CRASPs are encoded by at least two different gene families in *B. burgdorferi* strain ZST.

An increasing number of human pathogens express surface molecules that bind factor H and in some cases also FHL-1 such as the streptococcal M, Fba, and $\beta$ (also known as Bac or $\beta$C) (16–18, 28, 49); the pneumococcal PspC and Hic proteins (29–31) and the neisserial Por1A protein (32) have been identified. A factor H binding domain was identified previously for the borrelian BbCRASP-3 and other Erp proteins (35, 37, 39) as well as for the neisserial Por1A protein (32). Furthermore, a binding domain for both, factor H and FHL-1, was localized within the hypervariable region of the streptococcal M protein (17, 50). When compared with the previously identified factor H-binding motif of BbCRASP-3 (35), three putative factor H-binding regions (35) could be assigned to BbCRASP-1. However, removal of an 11-amino acid domain from the C terminus eliminated binding of both factor H and FHL-1, suggesting that the critical binding sites for factor H and FHL-1 do overlap and reside within the C terminus of BbCRASP-1.

The finding that BbCRASP-1 binds factor H and FHL-1 predominantly via SCR7 and possibly a linear determinant located in SCR20 of factor H has also been observed for M proteins of *S. pyogenes* (17–19, 50, 51, 52). However, binding of SCR5–6 and the C-terminal domain SCR20 to BbCRASP-1 was observed under denaturing conditions, i.e. Western blot and pepspot analysis. By using more physiological assays, i.e. surface plasmon resonance and ELISA, interactions of BbCRASP-1 with SCR5–6 and SCR20 were not observed. We therefore conclude that SCR7 is the critical recognition site for BbCRASP-1 under natural conditions. Binding domain(s) of factor H and FHL-1 for BbCRASP-1 are distinct for the complement regulatory domains located within domains SCR1–4. This is confirmed by the regulatory activity demonstrated for the bound proteins (Fig. 7). Despite the fact that FHL-1 bound more efficiently to BbCRASP-1, factor H exerted up to 6-fold stronger cofactor activity. This observation is explained by the higher decay-accelerating activity of factor H (53).

Particular outer surface proteins of *B. burgdorferi* are essential for their complement resistance (6, 9). Complement-resistant *B. burgdorferi* strains, which have lost plasmids during *in vitro* propagation, acquire susceptibility to complement-mediated killing (54–58). On the other hand, *B. burgdorferi* strain lacking plasmid lp54 seems to occur only rarely in nature (54). The fact that the main complement regulator-binding protein,
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BbCRASP-1, which is encoded by a linear plasmid lp54 encoded gene, adds to this contention. We therefore predict that bbCRASP-1 gene inactivation would dramatically increase virulence of the respective pathogen. Further studies of such gene-targeted mutants should provide additional insight into the mechanisms of complement resistance.

Data regarding the expression of BbCRASP-1 by spirochetes during the enzootic cycle are not available. We have shown recently that BbCRASP-1 is expressed by B. burgdorferi when seeding the tick gut but not during the course of experimental infection in mice (41). On the other hand, preliminary experiments suggest that BbCRASP-1 is expressed by spirochetes during natural infection and that sera from patients with various clinical manifestations of Lyme disease contain BbCRASP-1 specific antibodies.3 Thus it is tempting to speculate that Borrelia employ differential expression of BbCRASP-1 to readily escape the immune defense of the hosts.

In conclusion, we have identified a prominent outer surface protein of B. burgdorferi that binds to both human complement regulators, factor H and FHL-1. Our study suggests that BbCRASP-1 is a critical factor of B. burgdorferi in resistance against complement attack. Further studies on CRASP-1 molecules of different Borrelia species will help to elucidate immune escape mechanisms indispensable for persistence of the three human pathogenic Borrelia species.

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Complement Resistance of Borrelia burgdorferi Correlates with the Expression of BbCRASP-1, a Novel Linear Plasmid-encoded Surface Protein That Interacts with Human Factor H and FHL-1 and Is Unrelated to Erp Proteins

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